Response of Eastern and Formosan Subterranean Termites (Isoptera: Rhinotermitidae) to Borate Dust and Soil Treatments

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ABSTRACT Workers of the termites *Coptotermes formosanus* Shiraki and *Reticulitermes flavipes* (Kollar) were topically treated with disodium octaborate tetrahydrate, a fine-grain zinc borate, or boric acid (+1% magnesium stearate) powder; they were also exposed to sand treated with borates in an indirect exposure tunneling assay. Dust treatment with boric acid powder caused the most rapid mortality, with application of all three powders causing 100% mortality within 15 d. Treatment of 10–20% of the termite workers in test groups with borate dusts indicated that the toxicants are transmitted by grooming or trophallaxis (or both) to untreated individuals. However, less mortality occurred in groups of *C. formosanus* than in corresponding groups of *R. flavipes* workers when 10% of the workers were treated with disodium octaborate tetrahydrate or zinc borate powders. Both termite species readily penetrated sand containing 5,000, 10,000, or 15,000 ppm disodium octaborate tetrahydrate or zinc borate. In the 10-d test period, ≥5,000 ppm (or greater) disodium octaborate tetrahydrate and 15,000 ppm zinc borate in the sand elicited high mortality (85–93%) in *R. flavipes*. Responses of *C. formosanus* workers were more variable. Only 10,000 and 15,000 ppm zinc borate in the sand caused mortality (70–89%) significantly different from that in control groups. In both dust transmission and tunneling experiments, interspecific differences in grooming or tunneling behavior may cause reduced exposure of *C. formosanus* to the borates.

KEY WORDS Insecta, *Reticulitermes flavipes*, *Coptotermes formosanus*, boric acid

BORIC ACID AND BORON SALTS are effective termiticides (Randall et al. 1934, Reierson 1966, Williams & Amburgey 1987, Grace 1990b, Grace & Abdallay 1990), although the basis of their toxicity is not well understood (Williams et al. 1990). Sodium and zinc borates are used in wood preservation (Williams & Amburgey 1987, Barnes et al. 1989) and are currently of interest as soil insecticides (Grace 1990a), bait toxicants (D'Orazio 1982, Mori 1987, Grace 1990b), and termiticidal dusts (Grace & Abdallay 1990). Borate dusts could either be injected into termite galleries as was done with arsenicals (Randall & Doody 1934, Edwards & Mill 1986, Lin 1987), or used to contaminate foraging termite workers collected in traps such as those described by Su & Scheffrahn (1986) and Grace (1989). In a technique analogous to mark–release–recapture methodology, foragers coated with pesticide could be released back into the colony, and the toxicant would be passed among colony members by mutual grooming behavior (Grace & Abdallay 1990).

In the study described here, I examined the possible use of borates as soil treatments or insecticidal dusts to control Formosan subterranean termites, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). We compared the response of *C. formosanus* with that of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). Besides boric acid, we tested commercially available disodium octaborate tetrahydrate and a fine-grain zinc borate.

Materials and Methods

Termite Collection. Eastern subterranean termites, *R. flavipes*, were collected from traps consisting of corrugated cardboard rolled within short lengths of plastic pipe buried just below the soil surface at an urban site in Toronto, Ontario (Grace 1989). Formosan subterranean termites, *C. formosanus*, were collected from boards placed in traps on the soil surface at the Manoa campus of the University of Hawaii (Tamashiro et al. 1973). Tests were performed with workers (pseudergates older than the third instar as determined by size) in unlighted incubators at 27±0.5°C for *R. flavipes* and 29±0.5°C for *C. formosanus*. For both species, RH was ≈90%.

Materials. Two commercial borate powders were evaluated: disodium octaborate tetrahydrate (TIMBOR, 20.96% B, 1.20 Boric Acid Equivalents [BAE]), and a fine-grain (mean particle size, 3 μm) zinc borate (FIREBRAKE ZB-Fine, 14.92% B, 0.86 BAE) (United States Borax & Chemical Corporation, Anaheim, Calif.). Boric acid (17.48% B) with magnesium stearate (1%) added as an anticaking agent was also included in the dust treatment assays with *C. formosanus*. Other materials used in the assays differed slightly for the two termite species. *R.*

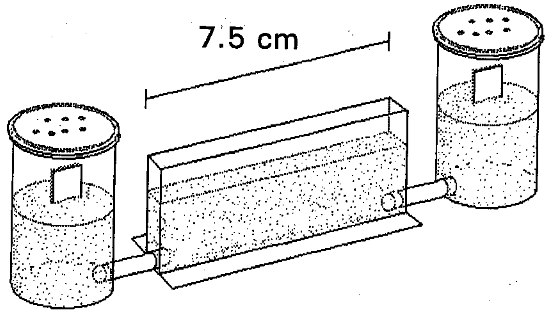

Fig. 1. Assay apparatus for measuring tunneling by *R. flavipes* and *C. formosanus* workers. Termites placed in one of the vials containing sand and cellulosic food must tunnel through the treated sand in the center tunneling arena (constructed of microscope slides) to reach the second vial. Short lengths of plastic tubing create an airspace between the untreated sand in the vials and the treated sand in the tunneling arena.

flavipes workers were kept in white silica sand (passing a U.S. no. 16-mesh screen) and fed strips of Whatman No. 1 filter paper (2 by 6 cm). *C. formosanus* workers were maintained in crushed coral sand (passing a U.S. no. 20-mesh screen) and fed short lengths of wooden tongue depressors (1.5 by 2.5 cm) (Puritan No. 25-705, Hardwood Products Company, Guilford, Maine).

Tunneling Bioassay. Termite workers were exposed to sand treated with borate in an assay described by Grace (1990a), which was similar to that described by Jones (1989, 1990) except that horizontal rather than vertical tunneling was recorded. This assay mimics indirect exposure to treated soil in a field situation, because termites are presented with the option of tunneling through treated sand to reach a second food source. The assay apparatus (Fig. 1) has three compartments: (1) a plastic vial containing untreated sand, termites, and cellulosic food; (2) a glass "sandwich", or tunneling arena, containing the treated sand; and (3) a second vial containing untreated sand and an additional food source. These are connected serially by 1-cm lengths of Tygon tubing. The sandwich-like tunneling arena consists of two glass microscope slides (2.5 by 7.5 cm) spaced 3–4 mm apart and secured in a horizontal upright position on one long edge by silicone rubber sealant (General Purpose Clear Sealant, Dow Corning Corp., Midland, Mich.) to a third flat glass slide as a base. The ends of the tunneling arena are sealed with plastic spacers and silicone caulking, with a 1-cm long Tygon tube at the base of each end of the sandwich leading into the base of one of the two 55-ml (15-dram) polystyrene vials (60 by 35 mm diameter). Each of these vials contains 10–15 g untreated sand, 2–3 ml water, and either a strip of filter paper or piece of wooden tongue depressor as food.

Each borate powder was thoroughly mixed in oven-dried sand to achieve compound concentrations of 5,000, 10,000 or 15,000 ppm (weight of compound per weight of sand). In previous assays, Grace (1990a) evaluated disodium octaborate tetrahydrate at 2,500 ppm, the rate recommended in experimental soil applications of this material in the United States (E. Docks, personal communication). As stated on labels for products used on termites, 2,500 ppm is equivalent to 1 lb disodium octaborate tetrahydrate (1 gallon TIM-BOR) per 10 square feet of soil (assuming penetration of 2 inches of soil).

The treated sand ($\approx$9 g) was poured into the tunneling arena, and 1.5 ml of water was added by pipette along the open top edge. The addition of water visibly moistened the sand to the base of the tunneling arena. The top edge of each tunneling arena was sealed with plaster of paris (to retain moisture), and 50 termite workers were placed in one of the adjacent vials. The vials were capped, a heated insect pin was used to pierce air holes in the caps, and the three-chamber apparatus was placed in the incubator. Each treatment was replicated six times. At 10 d, total tunneling distance in the arena and termite mortality were recorded and subjected to analysis of variance (ANOVA). Means were separated by the Ryan–Einot–Gabriel–Welsch multiple F test at $\alpha \leq 0.05$ (SAS Institute 1987). Tunneling distances could exceed the 7.5 cm length of the arena because of sinuous tunnels or multiple tunnels within a single sandwich. Percentage mortality data was transformed by the arcsine of the square root before analysis.

Dust Treatments. Toxicity of the borate powders to *C. formosanus* was determined with methods similar to those previously used with *R. flavipes* (Grace & Abdallay 1990). *Coptotermes formosanus* workers were coated with each powder by placing a group of 50 workers in a small (4.5 cm diameter) glass petri dish containing a thin layer of powder, gently shaking the dish, and then pouring the termites out onto a weighing paper placed in the center of a larger metal tray. After walking off of the weighing paper, the dust-coated termites were gently poured from the tray into a 45-ml polystyrene vial containing 10–15 g sand, 2–3 ml water, and either filter paper or wood as described above. The capped vials were maintained in an incubator. Mortality (including moribund individuals) was recorded at either 2, 4, or 8 d. Three groups of 50 workers from each of 3 field colonies were evaluated at each time interval (3 replicates $\times$ 3 colonies $\times$ 3 time intervals).

A second dust treatment assay was done with *C. formosanus* to determine whether the borate powders could be transmitted by grooming behavior to kill unexposed members of the group, as was demonstrated with *R. flavipes* (Grace & Abdallay 1990, Grace et al. 1990). In this test, groups of 80 *C. formosanus* workers were placed in vials prepared as described above, and allowed to acclimatize for 24 h in an incubator. Twenty (20% of each group) dust-treated workers were then added to each vial, and group mortality 15 d later was

Table 1. Tunneling and mortality of *R. flavipes* and *C. formosanus* after 10 d of indirect exposure to sand treated with borates

Compound	ppm[a]	*R. flavipes*		*C. formosanus*	
		Tunneling distance, cm[b]	% Mortality[b]	Tunneling distance, cm[b]	% Mortality[b]
Disodium octaborate tetrahydrate	5,000	8.27 ± 0.85a	85.33 ± 8.76ab	5.28 ± 1.61a	19.67 ± 2.39bc
	10,000	9.10 ± 1.38a	92.00 ± 6.53a	7.78 ± 0.52a	50.00 ± 17.46abc
	15,000	8.10 ± 0.60a	94.33 ± 2.03a	6.28 ± 1.10a	57.33 ± 14.37abc
Fine-grain zinc borate	5,000	9.05 ± 1.71a	52.33 ± 6.44c	4.68 ± 2.55a	32.33 ± 13.96abc
	10,000	8.88 ± 1.25a	69.00 ± 3.99bc	7.87 ± 1.57a	89.00 ± 6.17a
	15,000	8.50 ± 0.95a	93.33 ± 2.29a	7.05 ± 1.88a	69.67 ± 14.38ab
Control (water)		9.85 ± 0.97a	9.67 ± 2.60d	6.05 ± 0.87a	7.33 ± 1.84c

[a] Weight of compound to weight of sand. Powdered compounds were mixed dry in dry sand.
[b] Mean ± SEM of six groups of 50 termite workers. Means within each column followed by the same letter are not significantly different ($\alpha \leq 0.05$, ANOVA, Ryan–Einot–Gabriel–Welsch multiple F test [SAS Institute 1982]).

compared with that of completely dust-treated groups and control groups handled similarly (n = 6 groups of 100 workers per treatment).

A third dust treatment test was done with both *R. flavipes* and *C. formosanus* to determine the mortality caused by treatment of 10% of a group (5 of 50 workers) with either disodium octaborate tetrahydrate or fine-grain zinc borate powder. In this test, three 45-ml polystyrene vials were connected serially at the base by 1-cm lengths of Tygon tubing. The two end vials were prepared with sand, water, and food as in the tunneling assays; the center vial was left empty. Forty-five untreated termite workers were placed in the empty center vial, where they quickly moved through the Tygon tubes and initiated tunneling in the vials containing sand. These termites were allowed to equilibrate in the vials in an unlighted incubator for 24 h before five workers treated with dust were added to the center vial (n = 6 groups per treatment). Thus, the assay simulated introduction of borate-treated termites into a trap connected to an established gallery system. Termite mortality was recorded 15 d after introduction of the dust-treated workers.

Percentage mortality data was transformed by the arcsine of the square root and subjected to ANOVA. Means were separated by the Ryan–Einot–Gabriel–Welsch multiple F test at $\alpha \leq 0.05$ (SAS Institute 1987).

Results and Discussion

Neither disodium octaborate tetrahydrate nor zinc borate inhibited tunneling by *R. flavipes* and *C. formosanus* in treated sand, even at concentrations as high as 15,000 ppm (Table 1). However, a 10-d exposure to borate-treated sand caused significant mortality in *R. flavipes*. At 5,000 ppm of disodium octaborate tetrahydrate, *R. flavipes* mortality (mean ± SEM, 85.33 ± 8.76%) was similar to that (80.56 ± 3.38%) reported in a similar assay with an aqueus solution of this compound (Grace 1990a). Reduced mortality with more variation was noted with *C. formosanus*; only zinc borate at 10,000 and 15,000 ppm caused mortality significantly different from that of the controls. Penetration of the tunneling arena generally occured within 24 h and *C. formosanus* tended to excavate fewer and larger galleries than *R. flavipes*. This response may have reduced exposure of *C. formosanus* to the treated sand once the initial gallery was constructed. *C. formosanus* has also been observed to cover insecticide-treated soil with bits of masticated wood and filter paper (unpublished observations), suggesting that this species may be able to reduce exposure to the treated sand by lining its galleries. Subterranean termite colonies are characteristically lined with excreta (Lee & Wood 1971).

When *C. formosanus* workers were treated with borate dusts, the rates of mortality were similar to those observed with *R. flavipes* (Grace & Abdallay 1990), with boric acid causing the greatest mortality within the 8-d period (Table 2). The responses of the three colonies tested did not differ significantly after the second day. All three compounds resulted in 100% mortality of treated termites within 15 d (Table 3).

Introduction of treated workers (20% of the group) into groups of untreated termites led to significant mortality (Table 3), indicating toxicant transmission by mutual grooming behavior and possibly by cannibalism. All termites exposed to workers treated with boric acid died within the 15-d test period. Mortality from disodium octaborate tetrahydrate and zinc borate exposure was also significant, although less than that induced by boric acid. Even assuming that all topically treated individuals died, corrected mean percentage mortalities for indirectly exposed workers of 50.00 ± 5.57% for disodium octaborate tetrahydrate and 45.63 ± 4.62% for zinc borate greatly exceeded mortality in the control groups (16.50 ± 9.57%).

Treatment of 10% of the group with either zinc borate or disodium octaborate tetrahydrate powders resulted in much greater mortality with *R. flavipes* (70–98%) than with *C. formosanus* (19–28%) (Table 4). The similar rates of mortality noted

Table 2. Mortality of *C. formosanus* workers after coating with borate dusts

Compound	% Mortality[a] after		
	2 d	4 d	8 d
Boric acid[b]	6.22 ± 3.29ab	55.33 ± 7.33a	76.67 ± 5.06a
Fine-grain zinc borate	6.67 ± 1.25a	32.89 ± 4.70b	39.78 ± 6.36b
Disodium octaborate tetrahydrate	3.56 ± 1.44ab	27.33 ± 3.21b	59.11 ± 6.45b
Control	0.67 ± 0.33b	5.11 ± 1.01c	6.22 ± 1.43c

[a] Percent dead or moribund. Mean ± SEM of nine groups (three per colony) of 50 termite workers. Means within each column followed by the same letter are not significantly different ($\alpha \leq 0.05$, ANOVA of transformed data blocked by colony, Ryan–Einot–Gabriel–Welsch multiple *F* test [SAS Institute 1982]).
[b] 99% boric acid + 1% magnesium stearate.

with both species after complete dust treatments suggests that behavioral rather than physiological differences between the two species account for the reduced mortality of *C. formosanus*. Less contact among individuals (reducing grooming behavior), or a greater rate of tunneling (increasing abrasion of particles from the cuticle) could limit accumulation of a toxic dose by untreated workers.

The results of these and other (Grace 1990a) tunneling bioassays demonstrate that treatment of the soil with disodium octaborate tetrahydrate or zinc borate does not create a barrier to termite penetration, as is expected of conventional termiticides (Jones 1989, Smith & Rust 1990). Although treatment of cellulosic materials with high concentrations of disodium octaborate tetrahydrate can deter termite feeding (Williams & Amburgey 1987, Grace et al. 1990), borates generally fit the profile of slow-acting nonrepellent, or type 3 toxicants (Su et al. 1982). Current soil treatments around buildings with fast-acting or repellent termiticides have little effect on termite populations outside the narrow band of treated soil (Su & Scheffrahn 1988, Grace et al. 1989). However, passage of termite foragers through borate-treated soil could be expected to gradually reduce the termite population. My study indicates that borate treatment of a band of soil outside the primary perimeter treatment or around food sources such as stumps (Grace 1990a) may prove more effective with *R. flavipes* than with *C. formosanus*.

From studies with *R. flavipes*, Grace & Abdallay (1990) suggested that insecticidal dust treatments might be used to control subterranean termites in a toxic variation of the mark–release–recapture technique used to monitor colony foraging behavior and estimate population size (Su et al. 1984, Su & Scheffrahn 1988, Grace et al. 1989, Jones 1990). The results of my study support this approach with *R. flavipes*, but indicate that borate dust treatments are unlikely to reduce *C. formosanus* colony populations substantially unless a very large proportion of the foraging population can be contaminated. Mark–release–recapture estimates of *C. formosanus* colony foraging populations range from 1 to 10 million (Su et al. 1984, Su & Scheffrahn 1988, Tamashiro et al. 1980, Yates & Tamashiro 1990), and capture and treatment of 20% of such populations would be a formidable task. Treatment of recently established colonies, or of aerial colonies initiated by alates aboveground in buildings may be more practical. Use of adjuvants in dust treatments to increase adhesion of particles to the treated insects' cuticle could also help promote efficient delivery of toxicant to other colony members, and reduce the ratio of treated to untreated individuals needed to achieve control.

Table 3. Mortality in groups of *C. formosanus* 15 d after treatment of 20 or 100% of each group with borate dusts

Compound	% Mortality[a]	
	20% Treated	100% Treated
Boric acid[b]	100.00 ± 0.00a	100.00 ± 0.00a
Disodium octaborate tetrahydrate	60.00 ± 4.45b	100.00 ± 0.00a
Fine-grain zinc borate	56.50 ± 3.70b	100.00 ± 0.00a
Control[c]	—	16.50 ± 0.57c

[a] Mean ± SEM of six groups of 100 workers. Means followed by the same letter are not significantly different ($\alpha \leq 0.05$, ANOVA of transformed data, Ryan–Einot–Gabriel–Welsch multiple *F* test [SAS Institute 1982]).
[b] 99% boric acid + 1% magnesium stearate.
[c] Handled similarly but not treated.

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Table 4. Mortality in groups of *R. flavipes* and *C. formosanus* 15 d after treatment of 10% of each group with borate dusts

Compound	% Mortality	
	R. flavipes	*C. formosanus*
Fine-grain zinc borate	98.00 ± 1.26a	18.67 ± 2.51ab
Disodium octaborate tetrahydrate	70.00 ± 4.53b	28.00 ± 2.88a
Control	23.33 ± 3.37c	13.67 ± 3.52b

Mean ± SEM of six groups of 50 workers. Means within each column followed by the same letter are not significantly different ($\alpha \leq 0.05$, ANOVA of transformed data, Ryan–Einot–Gabriel–Welsch multiple *F* test [SAS Institute 1982]).

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