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ABSTRACT
In laboratory assays simulating field conditions, tunneling and mortality of *Reticulitermes flavipes* (Kollar) workers were evaluated in sand treated with aqueous solutions of formulated chlorpyrifos, isofenphos, and disodium octaborate tetrahydrate. Chlopyrifos and isofenphos were evaluated at concentrations of 500 ppm and 1000 ppm (weight of active ingredient / weight of sand), and disodium octaborate tetrahydrate at 2500 ppm and 5000 ppm. No tunneling was observed in sand treated with chlorpyrifos, and high termite mortality suggested vapor and/or high contact toxicity. Tunneling was initiated in isofenphos-treated sand, and termites subsequently died in the tunnels from contact toxicity. At both concentrations, termite mortality from isofenphos exposure was equivalent to that with chlorpyrifos, but the shorter tunnel length at the higher isofenphos concentration (1000 ppm) indicated a concentration-dependent rate of mortality. Greatest tunneling was observed in sand treated with disodium octaborate tetrahydrate. Low and variable mortality with this compound at 2500 ppm are attributable to its slow toxic action and, possibly, difficulty in obtaining an homogenous distribution. Tunneling was not inhibited at 5000 ppm, but mortality was comparable to that observed with 1000 ppm chlorpyrifos, although slightly less than with 1000 ppm isofenphos. Termiticides having different repellency/mortality profiles are potentially useful in termite control.

KEYWORDS: *Reticulitermes*, termite control, borate, disodium octaborate tetrahydrate, chlorpyrifos, isofenphos, Rhinotermitidae, Isoptera

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INTRODUCTION
In the past several years, restrictions on the use of organochlorine termiticides have led to the commercial development of organophosphate and pyrethroid pesticides for soil treatment to control subterranean termites (Isoptera: Rhinotermitidae). Other compounds, such as borates, are also under investigation. In order to use these new materials effectively, we need to understand the mechanisms by which they protect structures from termite attack. The relative value of repellency and toxicity in establishing a "chemical barrier" to termite infestation and the feasibility of using slow-acting pesticides in termite control are critical and controversial areas of concern (Su *et al.* 1982; Jones 1988, 1989).

This study was designed to evaluate eastern subterranean termite, *Reticulitermes flavipes* (Kollar), tunneling and mortality in indirect exposure to pesticide-treated sand. The laboratory assay mimicked field conditions adjacent to a termiticide-treated building. Termite workers provided with food (filter paper) were presented with the option of tunneling horizontally through treated soil to reach a second food source

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(decayed wood). Commercial formulations of the organophosphate termiticides chlorpyrifos and isofenphos and the inorganic compound disodium octaborate tetrahydrate were applied in aqueous solutions and termite tunneling and mortality were recorded after seven days.

MATERIALS AND METHODS

Eastern subterranean termites, *R. flavipes*, were collected in traps consisting of plastic pipe (ABS) containing rolled corrugated cardboard (Grace 1989). These traps were buried just below the soil surface at a site in downtown Toronto and a second site in the City of Scarborough. Termite populations at these two sites have been described by Grace *et al.* (1989). Termites collected while feeding on the corrugated cardboard were kept in plastic boxes in unlighted incubators at 27±0.5°C and 90±5% relative humidity. Bioassays using termite workers (undifferentiated individuals older than the third instar, as determined by size) were also performed in these incubators.

The test apparatus was similar to that used by Jones (1988, 1989, 1990), except that horizontal rather than vertical tunneling behavior was observed. This test simulates field conditions in that termites in a nesting chamber (plastic vial) provisioned with food (filter paper) have the option of tunneling through termiticide-treated sand (in a glass sandwich) to reach an alternate food source (decayed wood in a second vial). Thus, the apparatus consists of three compartments, connected serially with 1-cm lengths of tygon tubing. The first compartment is a 44.8-ml polystyrene vial (60 X 36-mm diameter) containing ca 10-g oven-dried white sand, a 2 X 6-cm strip of Whatman No. 1 filter paper as food, and 1.5-ml deionized water. This vial is connected to one end of a 7.5-cm long microscope slide "sandwich" by a 1-cm tygon tube inserted through a hole in the side of the vial, drilled below the surface of the sand. The sandwich consists of two microscope slides, spaced 3-4-mm apart, with the ends sealed with plastic spacers and silicone caulking. The sandwich is placed horizontally in an upright position, with the long edge secured by silicone caulking to a third flat glass slide, and filled with 9.5-g dry sand. A 1-cm tygon tube at the opposite end of the sandwich leads into another polystyrene vial identical to the first vial, except that it contains a 1-cm^2 block of red pine (*Pinus resinosa* Ait.) wood decayed by the fungus *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. rather than a strip of filter paper.

Three commercial pesticide formulations were included in this study. Two of these, Dursban TC^R (chlorpyrifos, Dow Chemical Canada and DowElanco)[2] and Pryfon 6^R (isofenphos, Chemagro Ltd. and Mobay Corporation)[2], are organophosphate termiticides registered in the United States, while the third, Tim-Bor^R (disodium octaborate tetrahydrate, US Borax & Chemical Corporation)[2], is a wood preservative in experimental use as a soil pesticide. Only Dursban TC^R is currently registered (under a temporary label) in Canada for termite control. Each of these three commercial products was mixed in deionized water and the aqueous solutions applied to achieve concentrations of active ingredient in the sand (weight of active ingredient / weight of sand) of 500 ppm and 1000 ppm with chlorpyrifos and isofenphos, and 2500 ppm and 5000 ppm with disodium octaborate tetrahydrate.

The sand in the center sandwich was treated with 1.5-ml deionized water (controls) or with the same quantity of an aqueous solution of one of the three pesticides, applied by pipette along the open top edge of the sandwich. In all cases, the solutions visibly moistened the sand to the base of the sandwich. The top edge of the sandwich was then sealed with plaster of paris, and 30 *R. flavipes* workers were placed in

[2]Mention of a company or trade name in this publication is for identification purposes only and does not constitute an endorsement by the University of Toronto or the University of Hawaii, or signify approval of any product to the exclusion of other comparable products.

the adjacent vial containing filter paper. Both vials were capped with polyurethane foam plugs, and the apparatus placed in the incubator.

Three groups (30 workers per group) from each of the two colonies were evaluated with each treatment. The total lengths of termite tunnels in the treated sand and termite mortality were recorded after 7 days. Tunnel lengths and percentage mortality were subjected to either *t*-test or analysis of variance (ANOVA) blocked by colony, and treatment means significantly different at the 0.05 level were separated by the Ryan-Einot-Gabriel-Welsch (REGW) multiple F test (SAS Institute 1987).

RESULTS AND DISCUSSION

In the control assays, tunneling and mortality after 7 days of *R. flavipes* workers from both colonies were equivalent (Table 1). Tunneling and mortality responses to the pesticide-treated sand also did not differ significantly between colonies.

Table 1. Tunneling and mortality of *Reticulitermes flavipes* workers from two colony sources after 7 days exposure to damp sand.[a]

Colony	Tunneling Distance (cm) (Mean ± SEM)	Percent Mortality (Mean ± SEM)
Scarborough	8.03 ± 0.32a	16.67 ± 3.85a
Toronto	10.50 ± 3.80a	16.67 ± 1.93a

[a]N = 3 groups of 30 workers. SEM = standard error of the mean. Means in the same column followed by the same letter are not significantly different (t-test, $\alpha \leq 0.05$).

No termite tunneling was detected in sand treated with chlorpyrifos at either 500 ppm (Table 2) or 1000 ppm (Table 3). This was in contrast to the significantly greater (and equivalent) tunneling in sand treated with isofenphos (3.12 ± 1.05-cm) or disodium octaborate (5.95 ± 1.19-cm) at the lower concentrations (Table 2). In similar assays with groups of 100 *R. flavipes* workers, Jones (1988, 1989) also noted negligible tunneling (less than 1-mm) in soil containing 500 ppm chlorpyrifos. As in that study, the high termite mortality observed here with chlorpyrifos suggests the possibility of vapor-phase as well as contact toxicity.

Tunneling was initiated in sand treated with both concentrations of isofenphos, indicating the absence of repellency or vapor-phase toxicity with this material. Termites were found dead in groups within the tunnels in the isofenphos-treated sand, resulting in high mortality (98.33 ± 1.67%) at 500 ppm comparable to that observed with chlorpyrifos (Table 1). Reduced tunneling in sand containing 1000 ppm isofenphos may indicate more rapid mortality from exposure to the higher pesticide concentration, rather than repellency (Table 3).

Table 2. Tunneling and mortality of *Reticulitermes flavipes* workers after 7 days exposure to sand containing lower concentrations of three pesticides.[a]

Pesticide	Tunneling Distance (cm) (Mean ± SEM)	Percent Mortality (Mean ± SEM)
Chlorpyrifos (500 ppm)	0 ± 0a	89.44 ± 2.91a
Isofenphos (500 ppm)	3.12 ± 1.05b	98.33 ± 1.67a
Disodium octaborate tetrahydrate (2500 ppm)	5.95 ± 1.19b	30.00 ± 10.58b

[a]N = 6 groups of 30 workers (3 replicates X 2 colonies). SEM = standard error of the mean. Means in the same column followed by the same letter are not significantly different (ANOVA, REGW multiple F test, $\alpha \leq 0.05$).

Table 3. Tunneling and mortality of *Reticulitermes flavipes* workers after 7 days exposure to sand containing higher concentrations of three pesticides.[a]

Pesticide	Tunneling Distance (cm) (Mean ± SEM)	Percent Mortality (Mean ± SEM)
Chlorpyrifos (1000 ppm)	0 ± 0a	91.11 ± 5.69ab
Isofenphos (1000 ppm)	2.03 ± 0.60a	97.22 ± 2.78a
Disodium octaborate tetrahydrate (5000 ppm)	10.35 ± 1.90b	80.56 ± 3.38b

[a]N = 6 groups of 30 workers (3 replicates X 2 colonies). SEM = standard error of the mean. Means in the same column followed by the same letter are not significantly different (ANOVA, REGW multiple F test, $\alpha \leq 0.05$).

Tunneling was most extensive in sand treated with disodium octaborate tetrahydrate. Although termite mortality increased at the higher concentration (5000 ppm), no concomitant decrease in tunneling activity was observed (Table 3). Mortality from exposure to 5000 ppm disodium octaborate tetrahydrate (80.56 ± 3.38%) did not differ significantly from that observed with 1000 ppm chlorpyrifos (91.11 ± 5.69%). However, 7-day mortality from exposure to 2500 ppm disodium octaborate tetrahydrate (30.00 ± 10.58%) was much less than that observed with 500 ppm of either chlorpyrifos or isofenphos (Table 2). Boron is a slow-acting toxicant, and 7-days may be an inappropriately short assay period. The large variation (indicated by the standard error) in mortality among replicates exposed to 2500 ppm disodium octaborate tetrahydrate suggests, though, that a more critical factor may be the difficulty in obtaining an homogenous distribution of this compound in the sand when it is applied in aqueous solution. Although impractical in the field, greater and more consistent mortality has been noted when the powdered compound was mixed into the sand prior to wetting (Grace, unpublished data). In these assays, as in field applications, application of the other pesticide solutions to the surface of the sand may also have resulted in concentration gradients, rather than an homogenous distribution of the active ingredients. However, no consistent pattern of tunneling was observed that would suggest this.

Repellency and toxicity of termiticides are factors that must be quantified and could be manipulated to achieve effective subterranean termite control (Su *et al.* 1982). Although not addressed in the present study, formulation with different emulsifiers might also add or detract from repellency. A pesticide, or concentration of pesticide, that does not permit penetration of the treated soil would seem to be the best choice for soil treatment immediately adjacent to building foundations. Repellency or high toxicity thus insures that the "chemical barrier" is not breached. However, remedial perimeter treatment of buildings actually appears to have very little effect on the termite populations outside that narrow band of pesticide-treated soil. Su and Scheffrahn (1988) and Grace *et al.* (1989), for example, have estimated large subterranean termite foraging populations at urban sites surrounding termiticide-treated buildings. Development of baits for subterranean termite control is one approach to reducing these foraging populations. However, passage of termite foragers through soil treated with a non-repellent slow-acting pesticide would also lead to mortality. In fact, soil treatment of a second outer zone around structures with a lesser concentration of the "barrier" termiticide, or with another non-repellent and slow acting termiticide, might be a complementary approach to current termiticide applications. This type of soil treatment around stumps and other exterior food resources might also be employed to convert these cellulosic resources into *in situ* baits.

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