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Horizontal transfer of boron by the Formosan subterranean termite (*Coptotermes formosanus* Shiraki) after feeding on treated wood

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Abstract

The goal of the present study was to determine whether *Coptotermes formosanus* Shiraki workers exposed to boron-treated wood were capable of transferring quantifiable amounts of boron to non-exposed nestmates. This effect is called horizontal transfer. Borates are not repellent to termites, nor do they cause rapid mortality, making them excellent candidates for the study of horizontal transfer. In the present study, *C. formosanus* workers were collected from field colonies maintained in Honolulu, Hawaii and dyed with Sudan Red 7B over a 7-day period. These termites then underwent a 3-day exposure period to one of two types of composite board, either an experimental formulation of zinc borate and anhydrous boric acid or an untreated composite board. Treated termites were placed with an equal number of untreated termites for either 5 or 10 days; sham-dyed donors and recipients were used to control for possible effects of the dye. Horizontal transmission of boron and toxic effects were assessed based on termite wet weight, percent survival, and boron content. Horizontal transfer of boron was noted at both 5 and 10 days over those levels reported in untreated, field-caught termites from the same colony (ca. 7 μg boron g^{-1} termites). After 5 days, boron content was elevated in both dyed donor and recipient termites (ca. 92 and 38 μg boron g^{-1} dyed termites, respectively), as well as in undyed donors and recipients (ca. 84 and 30 μg boron g^{-1} undyed termites). The same pattern was observed after 10 days in both dyed donor and recipient termites (ca. 61 and 46 μg boron g^{-1}, respectively) and undyed donor and recipient termites (59 and 24 μg boron g^{-1}, respectively). Increased boron content of recipient termites after exposure to donor termites (fed on treated wood) demonstrated horizontal transfer of boron.

Keywords: Aspen; boric acid; horizontal transmission; Isoptera; methylene diphenyl diisocyanate (MDI); Rhinotermitidae; waferboard.

Introduction

Horizontal transfer refers to the transmission of a compound of interest from one organism that has undergone exposure to the compound, the "donor", to a native member of a community, the "recipient". Understanding the horizontal transfer of non-repellent and slow-acting insecticides between donors and recipients is especially important for the management of social insects.

In a laboratory test, 100% mortality caused by the bait toxicant noviflumuron was found with a population of *Coptotermes formosanus* Shiraki. On the other hand, low mortality (35%) was observed following exposure to the liquid soil treatments fipronil and thiamethoxam, when the population was "split" into exposed and unexposed groups (Su 2005). The author interpreted these data as demonstrating the significance of horizontal transfer in the former case, and its reduction or absence in the latter. Shelton and Grace (2003) also demonstrated significant but minimal transfer of the non-repellent termiticides fipronil and imidacloprid between donors and recipients in laboratory tests. The pathway of horizontal transfer of insecticides is not always clear, though the concentration and exposure period of donors to recipients have been shown to have a significant impact on mortality in *C. formosanus* with the insecticide indoxacarb (Hu et al. 2005). Even in non-social insects, emetophagy, the ingestion of insecticide-induced regurgitate, has been shown to be an important mechanism of horizontal transfer of fipronil between adults and nymphs of the German cockroach [*Blatella germanica* (L.), Blattodea: Blattellidae] (Buczkowski and Schal 2001a). Insecticide formulation and the method of delivery also impact horizontal transfer: *B. germanica* transfers fipronil more effectively when it is ingested, rather than topically applied (Buczkowski and Schal 2001b). Horizontal transmission of pathogens has been noted as well, e.g., of *Beauveria bassiana* between larval cadavers and prepupae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), in the soil (Long et al. 2000).

Boron treatments of lumber and wood composites have a primary role in preventing degradation by bacteria and decay fungi (Grace 1997). In Hawaii, borate treatments are used extensively to protect against the Formosan subterranean termite, *C. formosanus* (Grace et al. 1992). Borates are not repellent to termites (Grace and Campora 2005; Campora and Grace 2007), nor do they cause rapid mortality (Grace et al. 1992; Ahmed et al. 2004; Kartal and Ayrilmis 2005), but whether horizontal transfer occurs has never been studied. Previous research (Gentz and Grace 2007) has established that zinc borate, disodium octaborate tetrahydrate, and anhydrous boric acid produce similar toxicity responses (e.g., levels of boron ingestion and mortality) in *C. formosanus*, and Su and colleagues (1994) determined the LD_{50} of boric acid as 721.3 μg boron g^{-1} in *C. formosanus* workers and 264.0 μg boron g^{-1} in *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae). Boron levels of 30.2 μg boron g^{-1} in termites maintained in the laboratory on untreated methylene diphenyl diisocyanate (MDI) composite boards for 5 days have been reported, in contrast to levels of 306.3 μg boron g^{-1} in termites exposed to composite boards treated with anhydrous boric acid in the same formulation used in this experiment (Gentz and Grace 2007).

The goal of the present study was to determine whether *C. formosanus* workers exposed to boron-treated wood were capable of transferring quantifiable amounts of boron to non-exposed nest mates.

Materials and methods

Termite collection

Coptotermes formosanus termites were collected in Douglas fir [*Pseudotsuga menziesii* Mirb. (Franco)] traps using a technique described by Tamashiro and colleagues (1973), from a field colony in Honolulu, Hawaii. Within 24 h of field collection, worker termites were mouth aspirated 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, USA), 30 ml distilled water, and a square piece of wood approximately 2.5 cm×2.5 cm×1 cm on an aluminum foil square centered on the sand as outlined in AWPA Standard E1-97 (American Wood Preservers' Association 2005). During the experiment, the jars were placed in a closed plastic box within an unlighted 28°C incubator at 68% relative humidity. The bottom of the box was lined with damp paper towels to maintain humidity. Untreated, field-caught termites collected at the same time from the colony were preserved by drying within 24 h of removal from the field to act as a baseline for the experimental treatment. Five replicates of 50 live termites were placed into an oven at 50°C for 3 h to dry. The dried termites were retained in 1.5 ml polypropylene microcentrifuge tubes and analyzed for boron content by inductively coupled plasma-atomic emission spectrometry (ICP-AES).

Dyeing termites with Sudan Red 7B

To definitively differentiate donor from recipient termites, groups of termite were fed filter paper dyed with the lipophilic dye Sudan Red 7B which, in turn, dyed the termites. Whatman No. 1 filter paper circles (55 mm diameter, 11 μm particle size range, Whatman International Ltd., Maidstone, UK) were dyed with Sudan Red 7B (Sigma Chemical Co., St. Louis, MO, USA) dissolved in acetone (HPLC grade, Fisher Scientific, Fairlawn, NJ, USA) to achieve a concentration of 1% of the filter paper weight as dye (adapted method of Lai et al. 1983). A 1-ml of dye solution was applied to each circle of filter paper and once the acetone had evaporated 2 circles of filter paper were placed in each Petri dish with an added 3 ml of distilled water to moisten the paper. Approximately 2 g of *C. formosanus*, collected as outlined above, were added to each Petri dish.

Termites were exposed to the dyed filter paper for 7 days in an unlighted 28°C incubator, monitored daily, and the dyed filter paper was replaced as it was consumed. After the 7-day dye exposure period, termites were transferred to the boron ingestion phase of the experiment.

Ingestion of boron

Dyed termites were then fed borate-treated timber for 3 days, a feeding period selected as one that would allow ingestion of measurable boron without resulting in high mortality or severe toxic effects (e.g., extreme weight loss and greatly reduced feeding) based on previous tests (Gentz and Grace 2007). Each of the replicates (donor or recipient) consisted of 2 g of *C. formosanus* workers, placed in a plastic jar with the wood sample (2.5 cm×2.5 cm×1 cm), prepared as described above. Experimental aspen composite waferboard with MDI resin (prepared by Rio Tinto Minerals) containing zinc borate/anhydrous boric acid (B_2O_3) (0.88% boric acid equivalent, or BAE, as determined by Rio Tinto Minerals) in a 60:40 ratio were prepared. Donor termites (1 replicate dyed, 1 undyed) were exposed to the borate-treated timber, and 2 replicates of recipient termites (2 dyed, 2 undyed) were exposed to untreated Douglas fir for 3 days. Before and after the experiment the wood blocks were oven dried at 40°C for 24 h, placed into a desiccator for 1 h, and weighed to measure wood consumption.

Test for horizontal transfer

The occurrence of horizontal transfer of boron was detected using termites previously dyed with Sudan Red 7B. To control for possible effects of the dye, replicates with "sham-dyed" termites (dyed, but not exposed to boron) were tested in addition to those replicates containing termites both dyed and exposed to boron. Five replicates of each treatment formed the basis for each of the 5- and 10-day transfer experiments; replicates for each test period (5 or 10 days) consisted of 100 donors and 100 recipients, with either the donors or recipients dyed. The 200 *C. formosanus* workers were placed into 15 cm Petri dishes with Whatman No. 2 (90 mm diameter, 8 μm particle size range, Whatman International Ltd.) filter paper circles moistened with 1 ml distilled water. Every 4th day, 500 μl of distilled water was added to prevent desiccation.

To determine whether the length of the exposure period had an impact on transfer, half (n=5) of the replicates were destructively sampled after 5 days, and the other half after 10 days. The number of live workers in each replicate was recorded, 50 termites from each replicate weighed, and the live termites were placed into an oven at 50°C for 3 h to dry. The dried termites were separated into groups of 50, retained in 1.5 ml polypropylene microcentrifuge tubes, and analyzed for boron content by ICP-AES.

Determination of boron content with ICP-AES

Termites were dried immediately after the destructive sampling of the replicate, or in the case of field-caught termites within 24 h of collection from the field. In this low-level elemental analysis technique, 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, USA) ICP-AES machine. The machine was calibrated with 0.0, 0.2, 0.5, and 1.0 ppm boron calibration standards in a 25% nitric acid matrix.

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 obtained as ppm boron, then converted to μg boron g^{-1} termites based on the average weight of the termites determined after the experiment, and divided by the number of termites in the assay (200 termites per jar) to calculate the amount of boron in each termite.

Statistical analyses

The mean wet weight per termite, percent survival, and boron content were analyzed for significant effects by analysis of variance (ANOVA) with SAS 9.1, and means were compared with the Ryan-Einot-Gabriel-Welsch Multiple Q-test (SAS Institute 2003). The effect of boron formulation on the other parameters measured in the assay was determined by a priori multivariate analysis of variance (MANOVA), an orthogonal contrast. The orthogonal general linear model (Proc GLM) test was applied to compare the mean boron content, wet weight, and survival rates of both dyed and undyed in donor and recipient termites after 5 and 10 days exposure. Data from untreated, field-caught termites from the same colony (collected and dried within 24 h of removal from the field) was the basis for comparison of the wet weight and boron content.

Results and discussion

Exposure to boron-treated timber for 3 days significantly decreased individual termite weight and survival. Dyed termite survival and their wet weight were decreased and their boron content was increased. These data support the hypothesis that boron ingestion at this level produces toxic symptoms that can lead to mortality in termites (Table 1). At both 5- and 10-day treatment levels, boron content was significantly higher in both donors and recipients compared to field-caught termites, which suggests that boron was ingested and transferred from donors to recipients (Table 1). The mean boron content of the recipient termites at 5 days, both undyed and dyed (29.5 and 38.4 μg boron g^{-1} termites, respectively), was comparable to the boron content of worker *C. formosanus* exposed only to untreated MDI (30.2 μg boron g^{-1}) as reported previously (Gentz and Grace 2007). After 10 days, boron levels in recipient termites were elevated compared to those of untreated field-caught termites. Additionally, the boron content of dyed and undyed donor termites, though decreased, was still elevated over boron levels reported in recipient termites (Table 1). Accordingly, initial boron exposure in donors (or lack of exposure in recipients) had a greater impact on boron content at the end of the test period than the effect of dyeing. This is a desirable result, since the dye was intended as a marker which should not influence the results.

An orthogonal contrast, a type of a priori MANOVA test, was performed to determine whether the boron formulation had an effect on wet weight (Table 2), survival

Table 1 Mean actual and observed boron content and wet weight for the 5- and 10-day exposure periods, plus data from untreated, field-caught *Coptotermes formosanus* termites provided for comparison; percent survival at 5 and 10 days for the experimental termites is also provided.

Exposure period	Caste	Experimental treatment	Mean actual boron content μg boron g^{-1} termites (mean[§]±SD)[#]	Individual boron content μg boron $termite^{-1}$	Mean individual wet weight mg $termite^{-1}$ (mean[§]±SD)[#]	Mean survival % (mean[§§]±SD)[#]
None	Worker	None	7.1a±0.64	0.0241	3.40a±0.1	N/A
(field-caught)	Soldier	None	7.3a±2.65	0.0257	3.52a±0.01	N/A
5 Day	Worker	Recipient, undyed	29.5bc±2.5	0.101	3.42a±0.1	96a±2
	Worker	Recipient, dyed	38.4cd±6.6	0.109	2.85bc±0.04	92a±11
	Worker	Donor, undyed	84.3f±8.1	0.278	3.30ab±0.1	91ab±3
	Worker	Donor, dyed	92.1f±4.1	0.243	2.64c±0.01	74c±4
10 Day	Worker	Recipient, undyed	24.2b±3.9	0.080	3.30ab±0.2	90ab±1
	Worker	Recipient, dyed	45.9d±8.2	0.118	2.58c±0.2	88ab±3
	Worker	Donor, undyed	58.9e±8.8	0.173	2.94bc±0.2	80bc±3
	Worker	Donor, dyed	60.5e±7.4	0.154	2.55c±0.1	55d±5

[§]Average of 5 independent replicates, 50 termites each.
[§§]Average of 5 independent replicates, 200 termites each.
[#]Values followed by the same letter are not significantly different from each other within columns (REGWQ ANOVA, P<0.01).

Table 2 Orthogonal contrast of the weight of dyed and undyed termites after 5 or 10 days of engaging in horizontal transfer of boron with data from untreated, field-caught termites is provided as a comparison.

			Untreated field-caught	5 Day				10 Day			
				Recipient		Donor		Recipient		Donor	
				Not dyed	Dyed	Not dyed	Dyed	Not dyed	Dyed	Not dyed	Dyed
5 Day	Recipient	Not dyed	NS								
		Dyed	†	**							
	Donor	Not dyed	NS	†	†						
		Dyed	†	†	†	†					
10 Day	Recipient	Not dyed	NS	NS	**	NS	†				
		Dyed	†	**	**	†	NS	†			
	Donor	Not dyed	***	†	†	*	**	*	**		
		Dyed	†	†	†	†	NS	†	NS	**	

Results from the orthogonal contrast are indicated as follows within columns: NS = no significant difference; *P<0.01; **P<0.05; ***P<0.001; †P<0.0001.

Table 3 Orthogonal contrast of survival in termites dyed with Sudan Red 7B and undyed termites after 5 or 10 days of engaging in horizontal transfer of boron.

			5 Day				10 Day			
			Recipient		Donor		Recipient		Donor	
			Not dyed	Dyed	Not dyed	Dyed	Not dyed	Dyed	Not dyed	Dyed
5 Day	Recipient	Not dyed								
		Dyed	NS							
	Donor	Not dyed	NS	NS						
		Dyed	†	†	†					
10 Day	Recipient	Not dyed	NS	NS	NS	†				
		Dyed	*	NS	NS	**	NS			
	Donor	Not dyed	†	**	**	NS	**	*		
		Dyed	†	†	†	†	†	†	†	

Results from the orthogonal contrast are indicated as follows within columns: NS = no significant difference; *$P < 0.01$; **$P < 0.05$; †$P < 0.0001$.

(Table 3), or boron content (Table 4) of the termites. At both 5- and 10-day treatment levels, significant decreases in weight were observed between the dyed and undyed donor and recipient termites (Table 2). Dyed donor termites also exhibited highly significantly lowered survival rates (Table 3). When comparing donor and recipient groups to untreated, field-caught termites, all of the experimental termites exhibited significantly increased boron content. Highly significant ($P < 0.0001$) increases in boron content were observed at both 5- and 10-day levels between the untreated field-caught, donor, and recipient groups. Thus, we suggest that boron content was elevated most in donor termites, and that boron was transferred to recipient termites, and that the boron levels of both donor and recipient termites was above those of untreated termites (Table 4).

Insecticides that can be horizontally transferred are especially useful in the control of pestiferous social insects. Success in this context has been described for the insecticides noviflumuron, fipronil, indoxacarb, and imidacloprid as cited in the introduction section. The most important characteristics of such insecticides are non-repellence and delayed toxicity to target insects and low vertebrate toxicity. Boron-treated timber fulfills these criteria and therefore boron-treated wood products are an important part of an integrated pest management strategy for combating termite attack.

Our goal was to determine whether boron can be horizontally transferred between worker *C. formosanus* termites and we conclude that such transmission does occur, even during short-term exposure. However, it is still unclear whether or not this transfer is able to cause significant mortality within a colony in the field, as was demonstrated in the laboratory. High mortality is potentially rendered irrelevant under field conditions because of the gradual avoidance behavior *C. formosanus* foragers exposed to borate treated lumber exhibited in the presence of other food sources (Campora and Grace 2007).

The boron content of undyed recipients after 5 and 10 days was significantly decreased from the observed boron content in donor termites. However, after 10 days dyed recipient termites had individual boron levels increased by a factor of approximately 1.5 compared to the undyed recipients. Dyed recipients after 5 and 10 days had comparable individual boron contents. Similar boron content was also recorded between dyed and undyed donors after 5 and at 10 days.

Thus, Sudan Red 7B may have exhibited a low level of interaction with the test compound in this study. The biologically active boric acid $B(OH)_3$ can complex with many compounds containing adjacent hydroxyl groups (see Gentz and Grace 2006); Sudan Red 7B, the lipophilic dye used as a marker in these experiments, contains such a moiety. The use of “sham-dyed” termites, or untreated termites that were not exposed to boron but were dyed, was implemented in this study to help control any possible interactions. The data show highly signifi-

Table 4 Orthogonal contrast of actual boron content in Sudan Red 7B dyed and undyed termites after 5 or 10 days of engaging in horizontal transfer of boron; data from untreated, field-caught termites is provided as a comparison.

			Untreated field-caught	5 Day				10 Day			
				Recipient		Donor		Recipient		Donor	
				Not dyed	Dyed	Not dyed	Dyed	Not dyed	Dyed	Not dyed	Dyed
5 Day	Recipient	Not dyed	**								
		Dyed	†	**							
	Donor	Not dyed	†	†	†						
		Dyed	**	†	†	**					
10 Day	Recipient	Not dyed	***	NS	**	†	†				
		Dyed	†	***	NS	†	†	†			
	Donor	Not dyed	†	†	†	†	†	†	**		
		Dyed	†	†	†	†	†	†	†	NS	

Results from the orthogonal contrast are indicated as follows within columns: NS = no significant difference; **$P < 0.05$; ***$P < 0.001$; †$P < 0.0001$.

cant differences in the parameters independently, whether termites were dyed or not. It can be safely concluded that the initial exposure to boron-treated timber in donors produced a greater impact on boron content than dyeing.

Delaplane and LaFage (1989) demonstrated that *C. formosanus* workers dyed with Sudan Red 7B had lower numbers of symbiotic protozoa, decreased feeding rates, and survivorship than non-dyed termites. Raina et al. (2004) showed that defaunation resulted in cannibalism by primary reproductives of *C. formosanus*. Accordingly, it is possible that termites defaunated by other means might also cannibalize nestmates, perhaps also as a result of the increased dose of insecticide they ingest. Lai et al. (1983) demonstrated that ingested Sudan Red 7B could be found in fat bodies, the gut, muscle, brain, and protozoa of *C. formosanus*. Boron compounds might also enter into several locations because of their wide biological distribution and interact with the dye. A topically applied dye, such as commercial gouache (Brunow et al. 2005), presents an alternative to ingested markers for short-term tests.

Boron has been shown to cause mortality in insects even after exposure at low concentrations. A 3-day exposure period enabled donor termites to transfer measurable quantities of boron to recipient termites. Even 10 days post-exposure, donor termites exhibited toxic symptoms, as seen through reduced weight, reduced survival, and elevated boron content. The boron-treated donor termites (3-day boron exposure) also showed a decreasing boron content between 5 and 10 days. We suggest that boron was being excreted or otherwise metabolized. The excretion may have affected the observed horizontal transmission, but does not influence the finding that transfer of boron occurred.

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