Effects of Exposure Duration on Transfer of Nonrepellent Termiticides Among Workers of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae)

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ABSTRACT The potential for transfer of nonrepellent termiticide toxicants between workers of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, was examined using two commercially available pesticide formulations and a simple donor-recipient model modified from current methods in the literature. Pesticides used were imidacloprid, formulated as Premise 75 WP, and fipronil, formulated as Termidor SC, in concentrations of 1, 10, and 100 ppm (weight of active ingredient/weight of sand) in sand. The results of the first experiment showed a significant increase in recipient mortality over control mortality when donor workers were treated with 100 ppm imidacloprid or 100 ppm fipronil. Although all three colonies studied were affected, one colony (colony 3) was affected to a significantly greater extent than the other colonies. This effect was not correlated with termite body size (dry mass). In a second study, recipient mortality was evaluated after exposure of donors to 1 ppm insecticide for 3, 6, 12, or 24 h. Recipient mortality indicated that these exposures did not consistently lead to lethal transfer of the insecticides.

KEY WORDS *Coptotermes formosanus*, nonrepellent termiticides, exposure duration

THE FORMOSAN SUBTERRANEAN termite, *Coptotermes formosanus* Shiraki, is an economically important urban pest species in the United States (Su and Scheffrahn 1990). Although recent advances have been made in the control of subterranean termite species, including *C. formosanus*, using bait technologies (Su 1994, Grace et al. 1996, Grace and Su 2000), soil insecticide treatment is widely used for prevention of structural infestations (Grace et al. 1993, Gahlhoff and Koehler 2001).

In the past several years, soil insecticides that kill termites but do not appear to repel them from entering treated soils have become popular alternatives to the use of more repellent materials as barriers to termite penetration. Lack of repellence and a delayed mode of action allow termites to freely move within treated soil before dying, and therefore may have a greater impact on the local termite population than repellent insecticides such as pyrethroids (Kard 2001). It has been theorized that the delayed mode of action of these new insecticides may allow transfer of the materials to occur from exposed to unexposed individuals (Kard 2001, Thorne and Breisch 2001). However, little information is available concerning the magnitude and mechanism of this transfer effect.

To address the question of whether transfer of insecticide occurs, and how such transfer is affected by insecticide concentration and duration of exposure, we used a simple donor-recipient model, in which termites exposed to treated sand are the donors, and unexposed nestmates are the recipients. By labeling the donors, we can separately evaluate donor and recipient mortality (Ferster et al. 2001). A similar approach was employed by Suárez and Thorne (2000) to study trophallaxis among termites.

The current study investigated transfer of two insecticide formulations considered to be relatively nonrepellent: fipronil (Termidor SC, Aventis Environmental Science, Montvale, NJ) and imidacloprid (Premise 75 WP, Bayer Corp., Kansas City, MO). Donor termites were exposed to various concentrations of insecticide in treated sand for various intervals. This included 24-h exposures at the lowest concentration of 1 ppm, to approximate field situations in which termites might be exposed to soil treated some years in the past. We hypothesized that even low concentrations of termiticide might result in successful transfer with extended exposures.

Materials and Methods

Termites. *Coptotermes formosanus* were collected from three field colonies, two of which were located on the Manoa Campus of the University of Hawaii

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Table 1. Generalized linear model results[a] and mean percent mortality (±SEM) of donor (D) and recipient (R) termites by colony after donor exposure to soil termiticide formulations at various concentrations (1, 10, or 100 ppm; first experiment)

Colony	Fipronil								Water	
	D 1	R 1	D 10	R 10	D 100	R 100	D Means	R Means	D	R
1	20.0 ± 11.5	5.6 ± 1.9	46.7 ± 13.3	5.6 ± 1.5	100 ± 0.0	28.1 ± 4.6	55.6 ± 12.8a	13.1 ± 4.0a	6.7 ± 6.7	7.4 ± 3.2
2	26.7 ± 6.7	4.2 ± 0.6	20.0 ± 11.5	5.6 ± 0.7	93.3 ± 6.7	33.7 ± 4.8	46.7 ± 12.5a	14.5 ± 5.0a	26.7 ± 17.6	4.2 ± 1.6
3	60.0 ± 11.5	13.3 ± 1.4	40.0 ± 20.0	8.1 ± 3.5	100 ± 0.0	54.0 ± 5.2	66.7 ± 11.1a	25.2 ± 7.5b	46.7 ± 6.7	9.5 ± 2.1
Means	35.6 ± 8.0a	7.7 ± 1.6y	35.5 ± 8.7a	6.4 ± 1.2y	97.8 ± 2.2b	38.6 ± 4.7z	-	-	37.8 ± 10.8a	8.4 ± 2.5y

	Imidacloprid							
	D 1	R 1	D 10	R 10	D 100	R 100	D Means	R Means
1	33.3 ± 13.3	3.9 ± 1.3	86.7 ± 6.7	8.8 ± 2.5	100 ± 0.0	47.4 ± 12.0	73.3 ± 11.1a	20.0 ± 7.7a
2	33.3 ± 17.6	4.2 ± 1.6	100 ± 0.0	7.4 ± 2.2	100 ± 0.0	50.5 ± 18.4	77.8 ± 12.2a	20.7 ± 9.2a
3	46.7 ± 6.7	12.6 ± 1.1	66.7 ± 6.7	9.8 ± 1.5	100 ± 0.0	86.7 ± 13.3	71.1 ± 8.2a	36.4 ± 13.2b
Means	37.8 ± 7.0a	6.9 ± 1.6y	84.4 ± 5.6b	8.7 ± 1.1y	100 ± 0.0b	61.5 ± 9.7z	-	-

[a] Identical letters following means are not significantly different at the $\alpha = 0.05$ level by Ryan-Einot-Gabriel-Welsch multiple Q-test. Colony comparisons are made only within the D means and R means columns. Concentration comparisons are made separately for each termiticide between column and water means for each mortality type [D (a, b) or R(y, z)].

(Honolulu, HI), and one from the Urban Garden Center in Pearl City, HI. All colonies were previously determined to be distinct from one another using mark-release-recapture methods (J.K.G. unpublished data). Termites were collected in Douglas fir, *Pseudotsuga menziesii* Mirb. (Franco), lumber using a trapping technique described by Tamashiro et al. (1973). Termites were freshly collected in two batches. The first batch was collected on the day that staining commenced for donor individuals (see below). The second group (recipients) was collected on the day that donors were exposed to treated sand and added into the jars containing the recipient termites (see details below).

Insecticides. Formulated insecticide concentrations were provided by the manufacturers. These were fipronil (Termidor SC, Aventis Environmental Science), and imidacloprid (Premise 75 WP, Bayer Corp.). Concentrates were diluted in distilled water as necessary to achieve active ingredient (a.i.) concentrations of 1, 10, or 100 ppm (as mass of active ingredient/mass of sand) in dry silica sand.

Donor-Recipient Model and Experimental Design. Each of the studies involved the use of a simple donor-recipient model of transfer between individuals. Stained workers were treated as described below, and considered "donors" that would transmit the toxicants to their untreated nestmates, the "recipients." Thus, we considered mortality of the recipients to represent movement of toxicants among the termites.

In both studies, termites destined to become donors were labeled by placing them for 10 d on 1% Sudan Red 7B (Sigma, St. Louis, MO) stained filter paper (Whatman #2, 9.0-cm diameter, Whatman International Ltd., Maidstone, England) with 1 ml of dH_2O added for moisture. After the staining period, recipient termites were collected from their respective colonies, counted into groups of 95 workers, and placed into glass screw-top jars (9.5 cm height × 9.0 cm diameter). The jars contained 150 g of autoclaved Silica sand (40–100 mesh; Fisherbrand, Fisher, Fair Lawn, NJ) with 30 ml of dH_2O added for moisture. Centered on top of the sand in each jar was a rectangle of aluminum foil (2.5 cm × 3.0 cm; Springfield Aluminum Foil, Certified Growers of California, Los Angeles, CA). The foil rectangle supported a single Douglas fir wafer (2.0 cm × 2.5 cm × 0.5 cm) as a food source for the termites.

Donor termites were counted into groups of 30 workers, and were exposed to 25 g of autoclaved silica sand (moistened with 6 ml of dH_2O) containing 0, 1, 10, or 100 ppm imidacloprid or fipronil by placing a single group of workers onto the surface of the sand in a plastic Petri dish (10.0 cm × 1.5 cm; Fisherbrand). Each colony by concentration combination was treated independently. Termites were allowed to remain on the treated sand for 1 h. Donor termites were then gently moved by brush and aspirator to a clean 9.0-cm diameter plastic Petri dish containing Whatman #2 filter paper, and allowed to walk for 30 min, during which any treated sand adhering to the termites was presumed to fall off and not contact the recipient termites later. After this cleaning period, donor termites were moved in groups of five to each glass jar according to colony affiliation. Therefore, 5% of the total group were donors, and 95% were recipients. There were three replicates of each treatment (three colonies by three concentrations by two compounds = 54 + 3 controls per colony = 63 U). Jars were held in an unlit 28 ± 1°C incubator at ≈90% RH, and the number and condition of donors was recorded daily. After a 2-wk incubation (day 15), the jars were disassembled and the number of surviving donors and recipients recorded.

In the second experiment, donor termites were exposed to either 0 or 1 ppm imidacloprid or fipronil, as described above. The length of exposure to the sand was different, however. Donors were exposed for 3, 6, 12, or 24 h to the treated sand before removal, cleaning, and addition to jars containing untreated nestmates. There were four replications (three colonies by two compounds by four durations = 24 + 4 controls per colony = 144 U) of the second experiment.

Statistical Analyses. The first experiment was designed as a 4 × 3 factorial design with pesticide concentration, colony, and their interaction as the factors examined. In that experiment, each insecticide was treated separately. Recipient and donor mortality (as percentage mortality) were transformed by the arcsine of the square root prior and separately subjected to generalized linear model analysis (Proc GLM; SAS Institute 1985), using the following parameters: concentration of insecticide, colony affiliation, and the colony by concentration interaction. The second experiment was also designed as a 4 × 3 factorial, using duration of exposure as the factor examined. For the second experiment, data were separated by insecticide treatment into donor and recipient mortality. These data were arcsine square root transformed and subjected to analysis of variance (ANOVA) (Proc ANOVA; SAS Institute 1985), comparing each treatment to the control (water) data. Means were separated using the Ryan-Einot-Gabriel-Welsch multiple Q-test (SAS Institute 1985) for both experiments.

Results

Mean donor and recipient mortality for each colony affiliation in the first experiment are presented in Table 1. Mean donor mortality ranged from 20 ± 11.5% for colony 2 (fipronil 10 ppm) to 100 ± 0.0% for colony 3 (both compounds at 100 ppm). Mean recipient mortality ranged from 3.9 ± 1.3% for colony 1 (imidacloprid 1 ppm) to 86.7 ± 13.3% for colony 3 (imidacloprid 100 ppm).

For imidacloprid in the first experiment, donor mortality was only significantly influenced by concentration (df = 3, 6; $F = 19.55$; $P < 0.0001$). Means separation indicated that donor mortality was significantly greater for 100 and 10 ppm imidacloprid than either controls or 1 ppm imidacloprid. Recipient mortality was significantly influenced by both colony and concentration (df = 3,6 and 2,6; $F = 32.46$ and 5.34; $P < 0.0001$ and $P = 0.0121$, respectively), but not by their interaction (df = 3, 6; $F = 2.01$; $P = 0.1040$). Recipient mortality was greater for 100 ppm imidacloprid than for any other imidacloprid exposure. Colony 3 recipient mortality was greater than either of the remaining colonies, which did not differ from each other.

For fipronil in the first experiment, donor mortality was also only significantly influenced by concentration (df = 3, 6; $F = 14.63$; $P < 0.0001$). Only 100 ppm fipronil significantly increased donor mortality above the control treatment. Again, recipient mortality was significantly influenced by both colony affiliation and concentration (df = 2, 6, and 3, 6; $F = 7.52$ and 45.42; $P = 0.0029$ and $P < 0.0001$, respectively), but not by their interaction (df = 3, 6; $F = 1.64$; $P = 0.1805$). Only 100 ppm fipronil significantly increased recipient mortality above that of the control treatment. Recipient mortality of colony 3 termites was significantly greater than either of the remaining colonies, which grouped together.

Mean mortality of both donor and recipient termites from the second experiment, separated by col-

Table 2. ANOVA results[a] and mean percent (±SEM) mortality of donor (D) and recipient (R) termites when donors are exposed to 1 ppm of fipronil (F), imidacloprid (I), or water (W) for different durations (second experiment)

Colony	F 3 h	F 6 h	F 12 h	F 24 h	I 3 h	I 6 h	I 12 h	I 24 h	W 3 h	W 6 h	W 12 h	W 24 h
1 D	90.0 ± 5.8b	100 ± 0.0b	100 ± 0.0b	100 ± 0.0b	55.0 ± 20.6a	100 ± 0.0b	40.0 ± 14.1a	79.9 ± 17.3a	10.0 ± 5.8a	30.0 ± 5.8a	50.0 ± 12.9a	35.0 ± 20.6a
1 R	12.9 ± 2.0y	13.7 ± 3.3y	5.3 ± 1.3y	4.2 ± 1.1y	6.8 ± 1.3y	7.6 ± 0.8y	9.2 ± 3.0y	8.2 ± 0.3y	8.4 ± 1.1y	8.7 ± 1.6y	9.5 ± 2.0y	6.6 ± 1.1y
2 D	95.0 ± 5.0b	100 ± 0.0b	100 ± 0.0b	100 ± 0.0b	75.0 ± 15.0b	55.0 ± 12.6a	55.0 ± 15.0a	35.0 ± 20.6a	15.0 ± 15.0a	5.0 ± 6.9a	20.0 ± 8.2a	50.0 ± 12.9a
2 R	4.0 ± 1.5y	6.1 ± 0.7y	5.3 ± 1.2y	26.6 ± 9.1z	5.3 ± 1.4y	5.8 ± 0.9y	4.7 ± 0.7y	5.8 ± 1.1y	6.9 ± 0.3y	6.1 ± 0.7y	3.2 ± 1.6y	3.2 ± 0.6y
3 D	100 ± 0.0b	100 ± 0.0a	100 ± 0.0b	100 ± 0.0b	85.0 ± 9.6a	90.0 ± 10.0a	90.0 ± 5.8a	100 ± 0.0b	70.0 ± 5.8a	75.0 ± 15.0a	45.0 ± 17.1a	60.0 ± 18.3a
3 R	4.7 ± 1.8y	10.5 ± 2.8y	22.9 ± 9.1y	9.0 ± 1.6y	6.6 ± 1.6y	5.8 ± 1.0y	9.7 ± 2.0y	17.9 ± 1.9z	5.0 ± 1.5y	8.2 ± 4.0y	8.4 ± 0.8y	8.7 ± 1.0y

[a] Identical letters following means are not significantly different at the $\alpha = 0.05$ level by Ryan-Einot-Gabriel-Welsch multiple Q-test. Comparisons are made between donor (a, b) and recipient (y, z) means for each colony and time with water.

Table 3. Mean (±SEM) dry masses (mg) of five termite samples taken from control treatments by exposure duration following each experiment

Experiment	Duration	Colony 1		Colony 2		Colony 3	
		Donors	Recipients	Donors	Recipients	Donors	Recipients
1	1	0.71 ± 0.03	0.82 ± 0.07	0.70 ± 0.06	0.79 ± 0.04	0.78 ± 0.03	0.84 ± 0.05
2	3	0.70 ± 0.04	0.84 ± 0.09	0.57 ± 0.03	0.72 ± 0.07	0.61 ± 0.03	0.76 ± 0.07
2	6	0.79 ± 0.04	0.71 ± 0.06	0.54 ± 0.08	0.73 ± 0.09	0.65 ± 0.03	0.75 ± 0.08
2	12	0.68 ± 0.07	0.78 ± 0.05	0.79 ± 0.05	0.85 ± 0.03	0.74 ± 0.05	0.77 ± 0.09
2	24	0.76 ± 0.03	0.87 ± 0.08	0.68 ± 0.05	0.71 ± 0.04	0.67 ± 0.04	0.88 ± 0.06

ony affiliation, are presented in Table 2. These results indicate that donor mortality varied among colonies, although overall the data indicate a significant increase in mortality in donors treated with both compounds at all treatment durations (Table 2). Recipient mortality is very different, however. For recipients, with only two exceptions, neither compound significantly increased mortality with any duration of exposure. The two exceptions, colony 2 with fipronil and colony 3 with imidacloprid, both at 24-h duration, indicate that there is some variation in susceptibility among the colonies used.

Discussion

The results of the first experiment indicate that there is transfer of these nonrepellent termiticides from exposed to unexposed Formosan subterranean termite workers. However, concentrations must be >10 ppm to have this effect in our bioassay. The rather small percentage (5%) of donors in our test populations (donors + recipients) certainly may have influenced these results. More donors might allow for insecticide transfer to occur at lower concentrations, or fewer donors might result in no lethal transfer at all. These are questions to address in future work.

Termite colony origin appeared to affect recipient mortality, with colony 3 recipients more susceptible to either insecticide than the other colonies. This was not directly related to differences in the physical size of the workers, because individuals in colony 3 were of equivalent size to those from the other colonies (Table 3). It is possible that intercolony differences in trophallaxis or grooming behavior may exist.

In the second experiment, mortality data for donors indicated that 1 ppm exposure to either insecticide for any of the durations was lethal. However, variability was again noted among the three colonies. Osbrink et al. (2001) have documented differences among subterranean termite colonies in susceptibility to a variety of soil insecticides, although imidacloprid was not included in that study. Our results also differ from those of Osbrink et al. (2001) in that they did not find significant differences in LT_{50} values among workers from different colonies exposed to fipronil.

With fipronil and imidacloprid, comparison of recipient mortality to control mortality did not indicate that donors exposed to 1 ppm of either insecticide were consistently successful in transferring a lethal concentration to recipient termites (Table 2), even with a 24-h insecticide exposure. Thus, it appears that termites must generally be exposed to concentrations >1 ppm in treated sand for lethal transfer to occur with their nestmates. However, it should be noted that two exceptions to this overall trend occurred with 24-h exposures, suggesting that an even longer exposure to low insecticide concentrations might lead to greater incidence of lethal transfer, so long as a sufficient number of donors remained alive for a long enough period to affect such transfer.

In summary, our results document lethal transfer of toxicants from exposed to unexposed *C. formosanus* workers when donors were exposed to 100 ppm imidacloprid or 100 ppm fipronil for 1 h. Extending the duration of exposure to 1 ppm of either compound to 24 h did not consistently result in significant mortality among the recipients. Questions that remain to be investigated include the mechanism of transfer (grooming, trophallaxis, or both), influences of caste (e.g., soldiers) and caste proportions on transfer, and the basis of differences in colony susceptibilities.

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References Cited

Ferster, B., R. H. Scheffrahn, E. M. Thoms, and P. N. Scherer. 2001. Transfer of toxicants from exposed nymphs of the drywood termite *Incisitermes snyderi* (Isoptera: Kalotermitidae) to unexposed nestmates. J. Econ. Entomol. 94: 215–222.

Gahlhoff, J. E., and P. G. Koehler. 2001. Penetration of the Eastern subterranean termite into soil treated at various thicknesses and concentrations of Dursban TC and Premise 75. J. Econ. Entomol. 94: 486–491.

Grace, J. K., J. R. Yates, M. Tamashiro, and R. T. Yamamoto. 1993. Persistence of organochlorine insecticides for Formosan subterranean termite (Isoptera: Rhinotermitidae) control in Hawaii. J. Econ. Entomol. 86: 761–765.

Grace, J. K., C.H.M. Tome, T. G. Shelton, R. J. Oshiro, and J. R. Yates III. 1996. Baiting studies and considerations with *Coptotermes formosanus* (Isoptera: Rhinotermitidae) in Hawaii. Sociobiology 28: 511–520.

Grace, J. K., and N.-Y. Su. 2000. Evidence supporting the use of termite baiting systems for long-term structural protection. Sociobiology 37: 301–310.

Kard, B. 2001. Gulfport studies stay the course. Pest Control 69: 30–33.

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

SAS Institute. 1985. SAS user's guide: statistics. SAS Institute, Cary, NC.

Su, N.-Y., and R. H. Scheffrahn. 1990. Economically important termites in the United States and their control. Sociobiology 17: 77–94.

Su, N.-Y. 1994. Field evaluation of a hexaflumeron bait for population suppression of subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 87: 389–397.

Suárez, M. E., and B. L. Thorne. 2000. Rate, amount, and distribution pattern of alimentary fluid transfer via trophallaxis in three species of termites (Isoptera: Rhinotermitidae, Termopsidae). Ann. Entomol. Soc. Am. 93: 145–155.

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.

Thorne, B. L., and N. L. Breisch. 2001. Effects of sublethal exposure to imidacloprid on subsequent behavior of subterranean termite *Reticulitermes virginicus* (Isoptera: Rhinotermitidae). J. Econ. Entomol. 94: 492–498.

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