Toxicity and Horizontal Transfer of Chlorantraniliprole in the Eastern Subterranean Termite

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ABSTRACT Toxicity and horizontal transmission of chlorantraniliprole were measured against field-collected eastern subterranean termites, *Reticulitermes flavipes* (Kollar). Chlorantraniliprole was highly toxic to termite workers in brief and continuous exposure assays across a range of concentrations from 5 to 100 ppm. All doses tested resulted in 100% mortality in the termites in 14 d. The effect of exposure route (topical, oral, or both) was investigated by exposing termites to treated substrate only, treated food only, or both. Results indicate that exposure route has no significant effect on chlorantraniliprole toxicity and demonstrate that chlorantraniliprole is highly active by feeding and contact. Results of feeding assays (paper consumption tests) demonstrate that as little as 5-ppm chlorantraniliprole applied to sand prevents termites from consuming cellulose that is in contact with the treated sand. Termites on untreated soil consumed 79 ± 3% of the available paper in 3 d, whereas termites on chlorantraniliprole-treated did not consume any paper before they became symptomatic and died. Results of transfer tests demonstrate that chlorantraniliprole is transferred efficiently among the termites. The rate and the level of secondary mortality in the recipient termites depend on both the concentration of chlorantraniliprole and the duration of exposure in the donors. Little secondary mortality was observed with the lowest dose of 5 ppm, which was effective at killing the donor termites, but insufficient to cause mortality in the recipient termites. In contrast, highly efficient transfer was observed with 25 and 50 ppm chlorantraniliprole. Both doses resulted in 100% mortality in the donors and the recipients at 21 d after exposing the recipients to the treated donors. These data demonstrate that chlorantraniliprole has dose-independent toxicity, delayed toxicity, and is readily transferred in eastern subterranean termites.

KEY WORDS chlorantraniliprole, eastern subterranean termite, horizontal transfer, insecticide, *Reticulitermes flavipes*

In natural ecosystems, subterranean termites are important decomposers of woody biomass and play an important role in the cycling of nutrients by feeding on cellulosic debris scattered on the forest floor (La Fage and Nutting 1978, Gentry and Whitford 1982, Sugimoto et al. 2000). During commercial development, the soil surface is cleared of all woody biomass and the normally scattered and ephemeral feeding sites are replaced with a single and highly-concentrated food source, the structure made from various wood-based materials. Human-built structures present the termites with the opportunity to colonize sites that can serve as highly stable and protected nesting, feeding locations, or both. Indeed, subterranean termites are of major economic importance as structural pests (Su 2002) and the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) is the most common and widely distributed termite pest species in the United States (Scheffrahn et al. 1988, Wang et al. 2009).

To protect the structures from termite damage, subterranean termites are controlled by applying liquid termiticides to the soil around the structures to create a chemical barrier. Barrier treatments first were developed in the 1940s and have changed very little since then (Lewis 1997). Depending on the type of construction, the treatments generally involve trenching and spraying the termiticide along the exposed soil and rodding and injecting the termiticide beneath concrete slabs. The treatments are labor-intensive and require a relatively large amount of insecticide to achieve the required concentration levels in the soil. Although the use of liquid termiticides has changed very little over the last century, tremendous progress has been made in the development of the chemicals used to perform the treatments. Historically, termite infestations were controlled with chlorinated hydrocarbons (e.g., aldrin, chlordane, heptachlor) and organophosphates (e.g., chlorpyrifos), both of which are no longer available. They were replaced by pyrethroids (e.g., bifenthrin, cypermethrin, permethrin) that were repellent to termites and thus prone to

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control failures (Smith and Rust 1990, Su et al. 1993, Gahlhoff and Koehler 2001). The use of pyrethroid insecticides has dramatically decreased with the introduction of various nonrepellent chemistries including chlorfenapyr, fipronil, and imidacloprid (Thorne and Breisch 2001, Shelton and Grace 2003, Hu 2005, Remmen and Su 2005), which became industry standards for termite control (Potter 2007). The newest termiticides include chemicals that are nonrepellent and pose less environmental risk (USEPA 2008). Principal among them is chlorantraniliprole registered for termite control as Altriset (EPA no. 352–829), the first termiticide to be registered as 'reduced risk' by the U.S. Environmental Protection Agency. Chlorantraniliprole belongs to the anthranilic diamide class of insecticides (Cordova et al. 2006, Lahm et al. 2007) and is a novel class of chemistry that targets and activates the insect ryanodine receptors in calcium channels (Coronado et al. 1994). This leads to rapid cessation of feeding, lethargy, partial paralysis, and regurgitation (Cordova et al. 2006).

Termite control exploits termite eusociality to deliver the insecticide from the site where it is applied (i.e., the treated structure) to the numerous and often far-away sites where the termites live and feed (i.e., satellite nests). Termites live in complex societies and engage in constant, close-contact interactions with other nestmates. These interactions include mutual grooming, cooperative nest construction, larval care, and most notably trophallaxis whereby foraging workers feed stages that do not or cannot forage on their own including early-instar larvae, reproductives, and soldiers (Buczkowski et al. 2007). When termites forage in termiticide-treated areas, they acquire the active ingredient and inadvertently share it with unexposed nestmates, a process known as horizontal transfer. Subsequently, horizontal transfer results in secondary mortality in situations where a lethal dose of the active ingredient is transferred from the exposed donor termites to the unexposed recipient termites. Horizontal transfer has been demonstrated previously with termiticides such as fipronil (Ibrahim et al. 2003, Saran and Rust 2007); hexaflumuron (Sheets et al. 2000); chlorfenapyr (Rust and Saran 2006); and imidacloprid (Osbrink and Lax 2003, Shelton and Grace 2003), although the relative contribution of horizontal transfer versus direct exposure to termiticides for overall termite control is still largely unknown and a subject of debate.

The goal of this project was to examine the toxicity and the horizontal transfer of chlorantraniliprole in the eastern subterranean termite, *Reticulitermes flavipes* under laboratory conditions. Previous studies on the effects of chlorantraniliprole in the eastern subterranean termites examined its comparative toxicity (Mao et al. 2011) and bioavailability in various soil types (Spomer et al. 2009). The specific questions addressed in this project included 1) the effect of exposure duration (short-term versus continuous) on termite mortality; 2) the effect of exposure route (oral, topical, or both) on termite mortality and behavior; 3) the effect of chlorantraniliprole on termite feeding and cellulose consumption; and 4) horizontal transmission of chlorantraniliprole.

Materials and Methods

Insects. Eastern subterranean termite, *Reticulitermes flavipes* colonies were collected on the campus of Purdue University, West Lafayette, IN by trapping within cardboard rolls inserted into the ground harboring termite colonies. The termites were brought into the laboratory and allowed to migrate into plastic containers with cellulose powder, moistened pine wood, and laboratory paper towels provided as food and harborage. Colonies were maintained at 25–27°C, >80% RH, and a photoperiod of 0:24 (L:D) h (constant darkness). Fifth- through seventh-instar workers were used in individual assay replicates. Colonies were checked and maintained weekly to ensure that moisture and food resources were not declining. Termites were kept in the laboratory for no >1 mo before testing.

Insecticide and Testing Substrates. Commercially formulated suspension concentrate of chlorantraniliprole was provided by DuPont Crop Protection (Wilmington, DE; DPX E2Y45–438; 200 SC, 20% [AI]). A 1,000-ppm stock was prepared by diluting 0.5 ml of concentrate in 99.5-ml distilled water. An appropriate amount of the stock was then further diluted in distilled water and 30 ml of the resulting mixture was mixed with 270 g of dried sand or soil. This yielded 300 g of soil or sand at the targeted concentration and soil moisture at 10%. For all tests using sand, dust-free, grade A, washed, and sterilized sand was used (Menards Inc., Eau Claire, WI). For all tests using soil, topsoil native to Indiana was collected in a natural area that had not received any pesticide treatments. The soil was transported to the laboratory and sieved with a number 10 sieve (2 mm opening) to achieve uniform particle size, autoclaved to kill any pathogens that might be harmful to the termites, and dried under ambient conditions. The soil was analyzed by CLC Labs (Westerville, OH) and was determined to have the following characteristics: clay loam (35% sand, 38% silt, and 27% clay); pH 7.6; and contained 6.7% organic matter.

Short-Term Versus Continuous Exposure to Chlorantraniliprole. Under field conditions, termites may be exposed to a termiticide for various amounts of time depending on the proximity to the treatment zone and other factors. Contact with the termiticide may be intermittent or continuous and the duration of exposure will affect the rate and the level of mortality in the termites. Such effects were investigated by exposing termites to chlorantraniliprole in short-term and continuous exposure assays. For short-term assays, 30 g of chlorantraniliprole-treated sand were placed inside of a 9-cm-diameter petri dish. Fifty termite workers were gently aspirated from a stock colony and added to the dish. Two doses of chlorantraniliprole were tested: 25 ppm and 50 ppm. The 50 ppm dose is based on the label rate being 0.05%, which once incorporated into the soil by following label directions, results in a concen-

tration of ≈50 ppm. The 25-ppm dose is representative of a soil concentration after a period of natural pesticide degradation. The termites were exposed to the treated sand for 2, 4, 6, 8, 10, or 12 h and then were transferred to a dish with moist untreated sand and food (paper towel). For continuous exposure assays, 30 g of chlorantraniliprole-treated soil were placed inside of a 9-cm-diameter petri dish and 50 termite workers were added to the dish. Four doses of chlorantraniliprole were tested: 5, 25, 50, and 100 ppm. The cover was replaced and the termites were returned to a dark incubator. The colonies were maintained at 25–27°C, > 80% RH, and a photoperiod of 0:24 (L:D) h (constant darkness). The termites were in direct, continuous contact with treated soil for the duration of the experiment. For both tests, the petri dishes were placed inside a humidified box and returned to a dark incubator. The number of moribund termites was assessed at 0.5, 1, 2, 4, 7, and 14 d after the initial treatment. Termites were categorized as moribund if they could not maintain an upright stance when prodded. Five replicates were performed for each concentration within each assay and control assays with termites exposed to untreated sand was performed as well.

Effect of Exposure Route on Termite Mortality and Behavior. Termites may obtain termiticides in one of three ways: by contact with the treated substrate (soil), by feeding on treated substrate, or the combination of the two. The contribution of each exposure route was investigated by exposing termites to treated substrate only, treated food only, or both. In the assay that used treated substrate, only 30 g of chlorantraniliprole-treated sand were placed inside of a 9-cm-diameter petri dish. Four doses of chlorantraniliprole were tested: 5ppm (0.15 mg [AI] per dish), 25 ppm (0.75 mg [AI]), 50 ppm (1.5 mg [AI]), and 100 ppm (3.0 mg [AI]). Fifty termite workers were aspirated from the colony and added to the dish. The termites were provided with an untreated food source consisting of moist filter paper disk (Whatman No. 1; 2 cm in diameter) placed on top of the sand. In the assay that used treated food only, 50 termite workers were added to a dish containing 30 g of untreated sand. A food source consisting of moist filter paper disk (Whatman No. 1; 2 cm in diameter) was treated with chlorantraniliprole at the four concentrations listed above and placed on top of the sand. In the assay that used treated substrate and treated food, 50 termite workers were added to a dish containing 30 g of treated sand and treated paper disk. The amount of chlorantraniliprole per dish was identical to the amount of chlorantraniliprole used in the other two assays. To achieve this, half the amount of chlorantraniliprole was used to treat the sand; the other half was used to treat the paper disk. In all assays, the cover was replaced after adding the termites and the dishes were returned to a dark incubator. The termites were in direct, continuous contact with the treated substrates for the duration of the experiment. The number of moribund termites was assessed at 0.5, 1, 2, 4, 7, and 14 d after the initial treatment. Termites were categorized as moribund if they could not maintain an upright stance when prodded. Five replicates were performed for each concentration and control assays with termites exposed to untreated sand and untreated food were performed as well.

Food Consumption by Termites Exposed to Chlorantraniliprole. Cellulose consumption by termites exposed to chlorantraniliprole-tread sand was determined. Thirty grams of chlorantraniliprole-treated sand were placed inside of a 9-cm-diameter petri dish. Four doses of chlorantraniliprole were tested: 5, 25, 50, and 100 ppm. Fifty termite workers were aspirated from a stock colony and added to the dish. The termites were not starved before the test. A 2- by 2-cm piece of brown paper towel was weighed to determine its initial dry weight and was placed on top of the treated substrate, one piece per dish. The cover was replaced and the termites were returned to a dark incubator. The termites were in direct, continuous contact with the treated sand for the duration of the test. Ten replicates were performed for each concentration and a control assay with termites exposed to untreated sand was performed as well. Paper consumption was estimated quantitatively 3 d after placing the termites on the treated substrate. This was accomplished by drying and reweighing the paper to determine its final weight.

Horizontal Transfer of Chlorantraniliprole. The horizontal transfer of chloroantraniliprole from live treated donor termites to live untreated recipient termites was examined. Transfer was measured with three different doses: 5, 25, and 50 ppm. Chlorantraniliprole was delivered to the donors by exposing them to chlorantraniliprole-treated sand or soil for 1, 2, or 4 h. To expose the donors, a plastic container (12 by 12 cm) was filled with treated sand or soil to the depth of 1 cm and the substrate was slightly compacted. The donor workers were aspirated gently from stock colonies by using a mouth aspirator and placed on top of the treated substrate. The donors were exposed en masse, in numbers that exceeded the number of individuals needed for the tests. After exposure, the appropriate number of donors was aspirated from the treated substrate and transferred to a petri dish (9 cm Ø) containing untreated recipients and a 8-cm-diameter disc of filter paper (Whatman) moistened with 0.5-ml water. Care was taken not to transfer any treated substrate along with the treated donors, and any sand or soil particles that became aspirated along with the termites subsequently were removed from the test dishes and discarded. To distinguish the donors from the recipients, the recipients were stained red by allowing them to feed on tissue paper containing 0.1% Sudan dye (Sigma-Aldrich, St. Louis, MO). The recipients were produced en masse by placing whole colonies on moist sand and provisioning them with the dyed paper in the absence of any other food. After staining (≈7–10 d), workers showing red dye were selected for all tests. A 1:1 ratio of 30 donors to 30 recipients was used in all tests. The lids were replaced and the dishes were placed inside of a dark incubator. Water was added to the filter papers as necessary. Mortality in the treated donors and the

untreated recipients was assessed at 1, 3, 7, 14, and 21 d after mixing the donors and the recipients. The termites were categorized as either presymptomatic (normal behavior), symptomatic, or dead. The termites were considered dead if they could not maintain an upright stance when prodded. Five replicates were performed for each concentration and exposure time combination and control assays with termites exposed to untreated sand or soil were performed as well.

Statistical Analyses. All data analyses were performed using SAS 9.2 statistical software (SAS Institute 2008). For all assays, comparisons among chlorantraniliprole doses (ppm), exposure times, exposure routes (oral versus dermal), test groups (donors versus recipients), and test substrates (sand versus soil) consisted of analysis of variance (ANOVA) (PROC GLM) on log transformed mean cumulative percent mortality. The level of significance was set at $\alpha = 0.05$.

Results

Short-Term Versus Continuous Exposure to Chlorantraniliprole. Results of short-term exposure tests indicate that both rates are highly effective and result in 100% mortality in the termites in 14 d (Fig. 1A and B). The effect of exposure time on the rate of mortality was highly significant (ANOVA, d.f. = 1, $F = 1122.2$, $P < 0.0001$). As expected, longer exposure times resulted in greater mortality in the termites. The effect of dose (ppm of chlorantraniliprole) was not significant (ANOVA, d.f. = 1, $F = 0.59$, $P = 0.444$) and both the 25 dose and the 50 ppm dose gave comparable results. Results indicate that 100% of the termites exposed to either dose became symptomatic within 12 h, even with the shortest exposure time of 2 h. The interaction between chlorantraniliprole dose and exposure time was also not significant (ANOVA, d.f. = 1, $F = 0.16$, $P = 0.686$). In the continuous exposure test (Fig. 1C), chlorantraniliprole concentration had no significant effect on the rate and the level of mortality (ANOVA, d.f. = 3, $F = 2.07$, $P = 0.109$), demonstrating that all four doses are equally effective. As expected, the effect of exposure duration was highly significant (ANOVA, d.f. = 1, $F = 232.5$, $P < 0.0001$) and increasing exposure time resulted in significantly higher mortality. The interaction between dose (ppm chlorantraniliprole) and time was not significant (ANOVA, d.f. = 3, $F = 0.32$, $P = 0.811$).

Effect of Exposure Route on Termite Mortality and Behavior. Termites may obtain chloroantraniliprole in one of three ways: by contact with the treated substrate (soil), by feeding on the treated substrate, and the combination of the two. Results indicate all routes of exposure are equally lethal to the termites (Fig. 2A–C). Exposure route had no significant effect on the level of mortality in the termites (ANOVA, d.f. = 2, $F = 2.39$, $P = 0.093$). Regardless of the exposure route, all termites became symptomatic within 12 h and all termites died within 7 d. Chloroantraniliprole concentration had a significant effect on termite mortality (ANOVA, d.f. = 3, $F = 2.76$, $P = 0.042$), however, the results for the lowest dose (5 ppm) and the highest dose (100 ppm) were comparable, suggesting that chlorantraniliprole is highly effective even at lower doses. Time since exposure had a highly significant effect on the level of mortality in the termites (ANOVA, d.f. = 1, $F = 755.65$, $P < 0.0001$), even though mortality was comparable for all three exposure routes in the early (0.5 and 1 d after exposure) and late (7 and 14 d after exposure) stages of the test. Finally, the interaction between time since exposure and dose (ppm) was not significant (ANOVA, d.f. = 3, $F = 0.52$, $P = 0.668$).

Food Consumption by Termites Exposed to Chlorantraniliprole. Results demonstrate that as little as 5 ppm prevents termites from consuming cellulose and the response is concentration independent (ANOVA, d.f. = 3, $F = 0.76$, $P = 0.325$). Termites on untreated soil consumed 79 ± 3% of the available paper in 3 d, whereas termites on chlorantraniliprole-treated soil did not consume any paper before they all became symptomatic and died.

Horizontal Transfer of Chlorantraniliprole. The results of transfer tests demonstrate highly efficient horizontal transfer from exposed donor termites to unexposed recipient termites. Mean donor and recipient mortality for tests carried out in sand and soil are presented in Tables 1 and 2 , respectively. In general, the level of horizontal transfer in all tests was dependent on three main factors: 1) chlorantraniliprole dose (ANOVA, d.f. = 2, $F = 145.48$, $P < 0.0001$); 2) exposure time in the donors (ANOVA, d.f. = 1, $F = 21.13$, $P < 0.0001$); 3) the test group (donors versus recipients) (ANOVA, d.f. = 1, $F = 13.74$, $P = 0.0002$); and 4) the substrate (sand versus soil) (ANOVA, d.f. = 1, $F = 6.70$, $P = 0.009$).

In assays using sand as the testing substrate (Table 1), donor mortality reached 100% at 21 d for all chlorantraniliprole concentrations with the exception of the lowest dose (5 ppm), which caused incomplete donor mortality at the 1- and the 2-h exposure. Mortality in the donors was significantly influenced by chlorantraniliprole concentration (ANOVA, d.f. = 2, $F = 27.89$, $P < 0.0001$); time of exposure (ANOVA, d.f. = 1, $F = 4.11$, $P = 0.044$); and the interaction of concentration and time (ANOVA, d.f. = 2, $F = 3.42$, $P = 0.034$). Donors exposed to chlorantraniliprole in sand efficiently transferred the insecticide to untreated recipients and mortality in the recipients reached 100% with both the 25 ppm and the 50 ppm doses, across all donor exposure times. Mortality in the recipients was significantly influenced by chlorantraniliprole concentration (ANOVA, d.f. = 2, $F = 14.67$, $P < 0.0001$) and time of exposure (ANOVA, d.f. = 1, $F = 8.96$, $P = 0.003$), but not the interaction of concentration and time (ANOVA, d.f. = 2, $F = 2.48$, $P = 0.086$). The lowest concentration tested (5 ppm) was the only dose that did not result in complete mortality in the recipients. The maximum level of mortality achieved in the recipients with the 5-ppm dose was 13 ± 6% when the donor termites were exposed to 5-ppm chlorantraniliprole for 4 h. Shorter exposure times in the donors (i.e., 1 and 2 h) resulted in little to no transfer.

Similar results were observed when the donor termites were exposed to chlorantraniliprole-treated soil

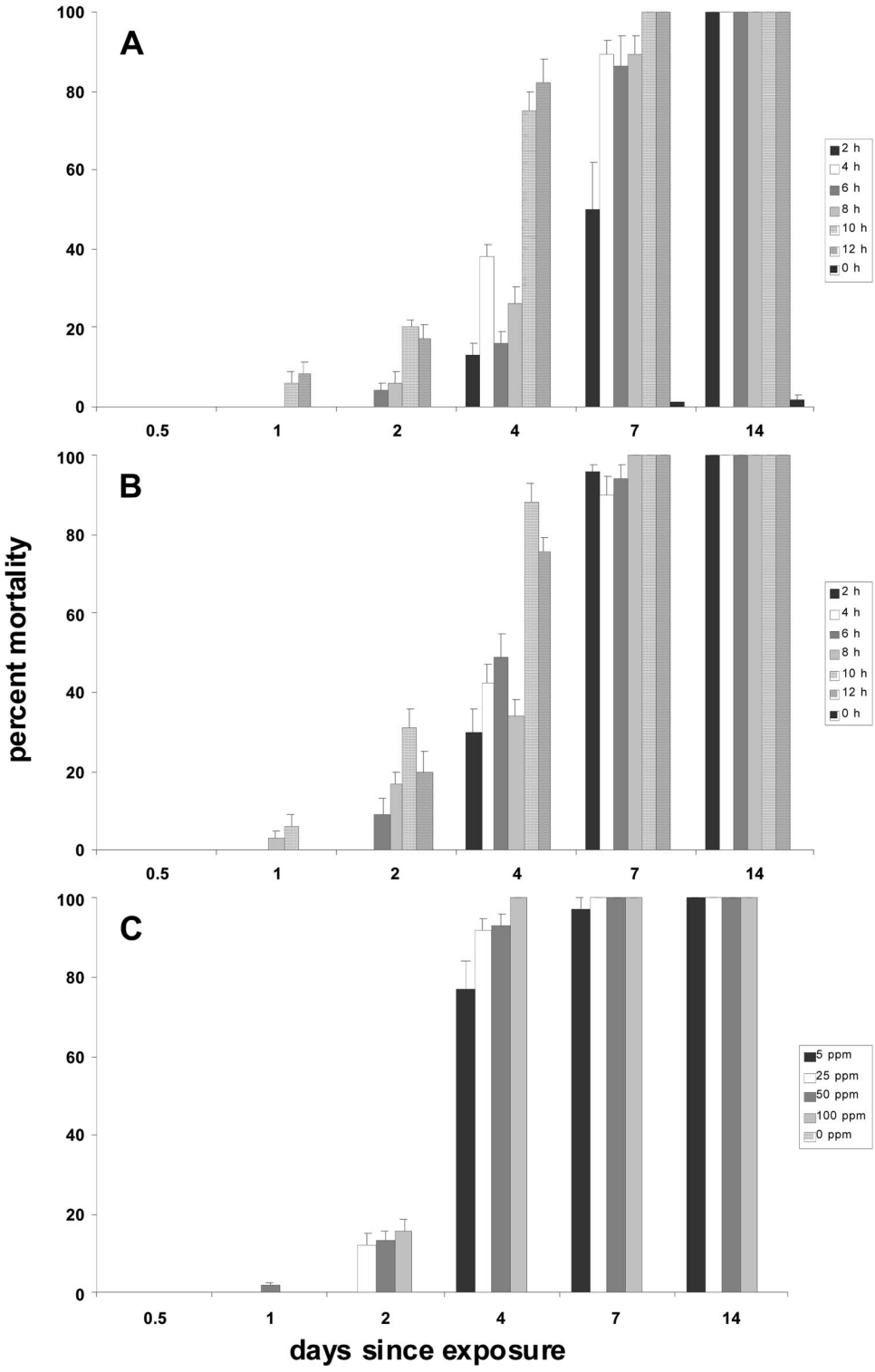

Fig. 1. Cumulative mean percent mortality (±SEM) in *R. flavipes* workers (A) after bihourly exposure to sand treated with 25-ppm chlorantraniliprole, (B) after bihourly exposure to sand treated with 50-ppm chlorantraniliprole, and (C) after continuous exposure to various concentrations of chlorantraniliprole in soil.

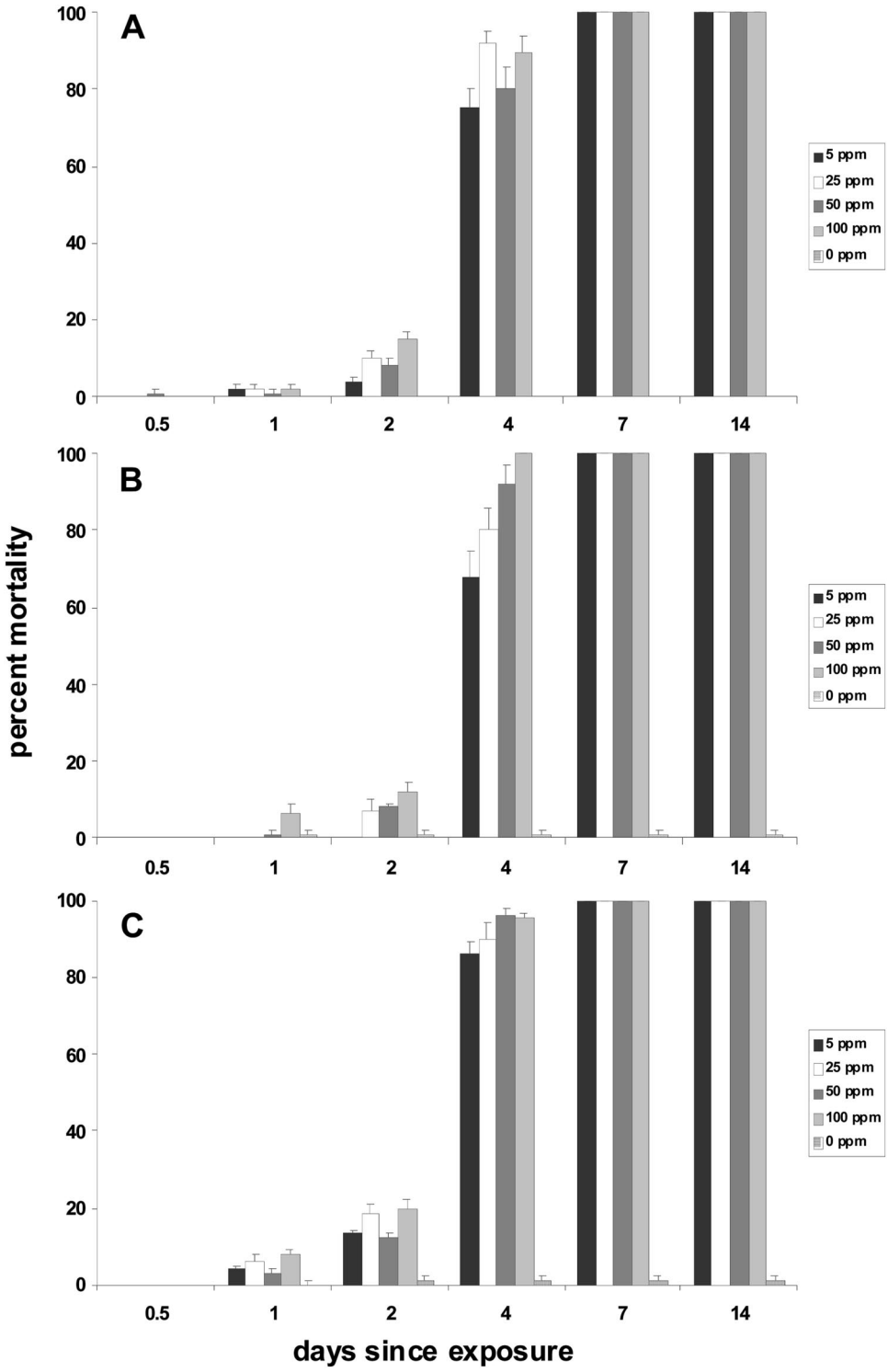

Fig. 2. Cumulative mean percent mortality (±SEM) in *R. flavipes* workers exposed to various concentrations of chlorantraniliprole in (A) sand only, (B) food only, and (C) sand and food.

Table 1. Mean cumulative percent mortality (±SEM) in donor and recipient termites exposed to three different concentrations of chlorantraniliprole-treated sand

Chlorantraniliprole concentration (ppm)	Exposure time in the donors (h)	Time since exposure of recipients to donors (d)				
		1	3	7	14	21
5	1	0 ± 0	0 ± 0	0 ± 0	6 ± 3	12 ± 4
		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
5	2	0 ± 0	0 ± 0	29 ± 8	33 ± 9	53 ± 9
		0 ± 0	0 ± 0	0 ± 0	9 ± 7	9 ± 4
5	4	0 ± 0	0 ± 0	65 ± 13	83 ± 9	100 ± 0
		0 ± 0	0 ± 0	4 ± 3	9 ± 4	13 ± 6
25	1	0 ± 0	22 ± 6	100 ± 0	100 ± 0	100 ± 0
		0 ± 0	5 ± 2	96 ± 3	100 ± 0	100 ± 0
25	2	1 ± 1	17 ± 7	100 ± 0	100 ± 0	100 ± 0
		1 ± 1	3 ± 2	97 ± 2	100 ± 0	100 ± 0
25	4	0 ± 0	30 ± 7	100 ± 0	100 ± 0	100 ± 0
		0 ± 0	17 ± 7	100 ± 0	100 ± 0	100 ± 0
50	1	0 ± 0	41 ± 9	100 ± 0	100 ± 0	100 ± 0
		0 ± 0	15 ± 9	100 ± 0	100 ± 0	100 ± 0
50	2	0 ± 0	37 ± 8	100 ± 0	100 ± 0	100 ± 0
		0 ± 0	24 ± 6	100 ± 0	100 ± 0	100 ± 0
50	4	0 ± 0	49 ± 7	100 ± 0	100 ± 0	100 ± 0
		0 ± 0	11 ± 6	100 ± 0	100 ± 0	100 ± 0
0 (control)	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		1 ± 0	1 ± 0	3 ± 3	3 ± 3	3 ± 3

Within each cell, top row is mortality in the donors, bottom row mortality in the recipients.

(Table 2). Mortality in the donors was significantly influenced by chlorantraniliprole concentration (ANOVA, d.f. = 2, $F = 16.98$, $P < 0.0001$); time of exposure (ANOVA, d.f. = 1, $F = 4.11$, $P = 0.044$); and the interaction of concentration and time (ANOVA, d.f. = 2, $F = 3.42$, $P = 0.034$). The 25 and 50 ppm doses resulted in 100% mortality in the donors at 21 d. Donor mortality with the 5-ppm dose ranged from 5 ± 3% to 93 ± 5% depending on the exposure duration. Mortality in the recipients was significantly influenced by chlorantraniliprole concentration (ANOVA, d.f. = 2, $F = 18.22$, $P < 0.0001$), but not by time of exposure (ANOVA, d.f. = 1, $F = 1.28$, $P = 0.259$) or the interaction of concentration and time (ANOVA, d.f. = 2, $F = 0.62$, $P = 0.538$).

Discussion

The eastern subterranean termite, *Reticulitermes flavipes* is highly susceptible to chlorantraniliprole with a 2-d LD_{50} of 2.13 ng per worker (Spomer et al. 2009) and a 7-d LD_{50} of 0.98 ng per worker (Mao et al. 2011). Termites affected by chloroantraniliprole become symptomatic within hours and display an array of unique behavioral symptoms. One of the first symptoms of exposure is the cessation of feeding, most

Table 2. Mean cumulative percent mortality (±SEM) in donor and recipient termites exposed to three different concentrations of chlorantraniliprole-treated soil

Chlorantraniliprole concentration (ppm)	Exposure time in the donors (h)	Time since exposure of recipients to donors (d)				
		1	3	7	14	21
5	1	0 ± 0	0 ± 0	0 ± 0	1 ± 1	5 ± 3
		0 ± 0	0 ± 0	0 ± 0	0 ± 0	2 ± 1
5	2	0 ± 0	0 ± 0	1 ± 1	8 ± 3	17 ± 6
		0 ± 0	0 ± 0	1 ± 1	3 ± 1	9 ± 2
5	4	2 ± 1	2 ± 1	37 ± 5	48 ± 8	93 ± 5
		0 ± 0	0 ± 0	3 ± 2	5 ± 3	12 ± 5
25	1	0 ± 0	0 ± 0	51 ± 11	87 ± 8	100 ± 0
		0 ± 0	0 ± 0	11 ± 4	14 ± 6	45 ± 6
25	2	0 ± 0	9 ± 6	100 ± 0	100 ± 0	100 ± 0
		0 ± 0	1 ± 1	100 ± 0	100 ± 0	100 ± 0
25	4	1 ± 0	15 ± 7	100 ± 0	100 ± 0	100 ± 0
		1 ± 1	3 ± 1	100 ± 0	100 ± 0	100 ± 0
50	1	0 ± 0	11 ± 6	100 ± 0	100 ± 0	100 ± 0
		0 ± 0	0 ± 0	89 ± 5	100 ± 0	100 ± 0
50	2	0 ± 0	7 ± 3	100 ± 0	100 ± 0	100 ± 0
		0 ± 0	2 ± 1	88 ± 5	100 ± 0	100 ± 0
50	4	1 ± 1	8 ± 4	100 ± 0	100 ± 0	100 ± 0
		1 ± 1	5 ± 3	95 ± 5	100 ± 0	100 ± 0
0 (control)	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Within each cell, top row is mortality in the donors, bottom row mortality in the recipients.

likely because of the inactivation of large mandibular muscles in the termite workers. Second, affected termites show a very characteristic grouping behavior whereby the symptomatic individuals aggregate in a cluster, cease moving, and remain inactive until they die. Other symptoms include a pronounced reduction in walking, trail following, grooming, and tunneling. Chlorantraniliprole is unique in that it can be classified as both a fast-acting and a slow-acting insecticide. It is fast-acting because it produces symptoms within hours and quickly affects normal termite behaviors such as tunneling, feeding, and grooming. However, the affected termites do not die right away, but enter a prolonged symptomatic stage where the affected individuals remain inactive, but alive for 3–5 d.

In the current study, chlorantraniliprole was toxic to the termites in both brief and continuous exposure tests and resulted in 100% mortality in the exposed termites. In brief exposure assays, using chlorantraniliprole-treated sand as the substrate, mortality in the termites reached 100% for all exposure times ranging from 2 to 12 h. Continuous exposure assays that used soil as the testing substrate were designed to examine the toxic properties of chlorantraniliprole under conditions that more closely reflected natural field conditions. Interestingly, no relationship was observed between chlorantraniliprole concentration and termite mortality. The rate of mortality was slightly delayed with the lowest dose (5 ppm), but was not significantly different from the higher doses of 25, 50, and 100 ppm.

Termites encountering insecticide-treated areas may obtain the active ingredient in one of three ways:1) by contact with the treated substrate (soil), 2) by feeding on the treated substrate, or 3) the combination of the two. Consequently, the toxicity of termiticides may be affected by the route of entry (dermal versus oral) and the oral route is thought to be the primary route of exposure for soil-applied termiticides (Forschler 2009). The oral route is highly effective because the termites can acquire the active ingredient in a variety of ways: 1) feeding directly on the treated substrate, 2) carrying contaminated soil particles used to construct foraging tunnels, or 3) feeding on nestmates that have died as a result of insecticide treatments (cannibalism). However, results from the current study demonstrate that exposure route has no significant effect on the toxicity of chlorantraniliprole. Mortality rate in the termites exposed to the combination treatment was slightly higher relative to mortality in either the oral or the dermal treatment, but the difference was not statistically significantly. Regardless of the exposure route, all termites became symptomatic within 12 h and all termites died within 7 d. A similar effect was observed for fipronil, whereas other insecticides were more toxic orally (imidacloprid) or dermally (chlorfenapyr and indoxacarb) (Forschler 2009). Similar results were also observed in the German cockroach, *Blattella germanica* (L.), where the horizontal transfer of fipronil was affected by the delivery route and the oral route was the most effective for transferring fipronil from treated donors to untreated recipients (Buczkowski and Schal 2001). The lack of observed difference between the individual routes and the combination route may perhaps be attributed to the fact that it is extremely difficult to separate the two routes because of natural termite behaviors. This is because termites tunneling through the treated soil inadvertently ingest some insecticide as they handle soil particles with their mandibles. Similarly, termites feeding on a treated food source come into physical contact with the food and receive some dermal exposure. Another factor contributing to the lack of observed difference is the fact that chlorantraniliprole concentration in all assays was equal as the dose in the combination assay was split equally between the substrate (sand) and the food. Assays on the effect of exposure route also revealed that chloroantraniliprole concentration had no effect on the level of mortality when the termites were exposed in sand only, food only, or the combination treatment. Within each treatment the rate and the level of mortality were comparable for doses ranging from 5 to 100 ppm. This further confirms the results from the short-term and continuous exposure assays and demonstrates that chlorantraniliprole is highly effective even at lower doses.

Paper consumption assays revealed that as little as 5 ppm prevents termites from consuming cellulose and the response is concentration independent. All four doses tested completely prevented the termites from consuming any paper, whereas the termites in the control test consumed almost 80% of the available paper in 3 d. Termites exposed to chlorantraniliprole-treated sand became symptomatic within hours as the insecticide causes the contraction of muscles, likely also affecting the large mandibular muscles in the termites, and results in the cessation of feeding and lethargy (Cordova et al. 2006). This result suggests that applications of chlorantraniliprole are highly effective in stopping termite feeding on treated structures and preventing further damage.

Chlorantraniliprole was transferred efficiently among the termites, and the rate and the level of secondary mortality in the recipient termites depended on both the concentration of chlorantraniliprole and the duration of exposure in the donors. Little secondary mortality was observed with the lowest dose of 5 ppm, which was effective at killing the donor termites, but insufficient to cause mortality in the recipient termites. In contrast, highly efficient transfer was observed with the higher doses of 25 and 50 ppm, with no significant difference between the doses. Both doses resulted in 100% mortality in the donors and the recipients at 21 d after exposing the recipients to the donors. Transfer was more efficient when the donors acquired chlorantraniliprole from sand versus soil, although the difference between the substrates was not statistically significant. Different treatments and substrates can affect the speed and efficacy of termiticides (Spomer et al. 2009, Mao et al. 2011) and soil typically lowers the speed of action because the organic matter in the soil binds the insecticide and makes it less available to the target insect. In addition, other soil

factors such as texture (percentage of clay and sand) and pH affect the fate and biological availability of insecticides (Gold et al. 1996, Spomer and Kamble 2011). However, such effects generally are observed only when the termiticide concentration falls below the label rate, and not at the label rates (Henderson et al. 1998). The organic matter content for the soil used in the current study was 6.7%, which is slightly higher that that reported in other studies where the organic matter concentration ranged from 0 to 4% (e.g., Gold et al. 1996, Spomer and Kamble 2011, Henderson et al. 1998). Despite the higher organic matter content chlorantraniliprole performed well and resulted in 100% or nearly 100% mortality depending on the rate. Indeed, Tables 1 and 2 demonstrate that there was little reduction in lethal transfer between a very low organic matter content substrate (sand) and a very high organic matter content substrate (soil) and only at the lowest concentrations. Termites exposed to chlorantraniliprole show a characteristic grouping behavior whereby the affected individuals cease moving and aggregate in a cluster. It is unclear whether the grouping behavior affects the magnitude of transfer. On the one hand, the grouping behavior might promote horizontal transfer as the increased proximity increases contact among individuals and creates opportunities for transfer. However, the lack of movement is thought to have a counteractive effect as it may limit exposure to chlorantraniliprole residues.

In summary, our tests demonstrate that chlorantraniliprole has dose-independent toxicity and delayed toxicity in eastern subterranean termites. Furthermore, it is efficiently transferred in termite colonies and causes high secondary mortality. Questions that remain to be answered include the mechanisms of transfer (grooming, trophallaxis, or both); radiotracer studies to determine the precise amounts of chlorantraniliprole acquired by the donors and transferred to the recipients; influence of caste (including reproductives) and caste proportion on transfer; and further analytical and behavioral studies to determine the importance of the unique symptoms of chlorantraniliprole exposure on its toxicity and transfer.

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