Horizontal Transfer of Insecticides in Laboratory Colonies of the Argentine Ant (Hymenoptera: Formicidae)

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ABSTRACT Five insecticides used by urban pest management professionals for ant control and three experimental insecticides were tested to determine whether these insecticides were horizontally transferred among individuals in colonies of Argentine ants, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae). Ants were exposed to insecticide-treated sand for 1 min and then placed in a colony of untreated ants. Ants exposed to 20 and 40 ppm fipronil readily transferred the insecticide to other individuals in the colony, resulting in high mortality. Most of the transfer and subsequent mortality occurred within 4 d after exposure to treated ants. The other insecticides were not transferred, and ants exhibited mortality rates similar to that of the controls. Experiments in large foraging arenas demonstrated that necrophoresis was an important behavior facilitating the horizontal transfer of fipronil. When ants contacted contaminated corpses in the process of removing them to refuse piles, they received a lethal dose of fipronil and subsequently died. Fipronil-contaminated dead ants that were placed in the vicinity of the nest resulted in significantly higher mortality than did corpses placed in a distant foraging arena (30 cm away). Most of the dead ants accumulated in the vicinity of the nest rather than in the foraging arena, workers retrieving dead ants to refuse piles from the foraging arena. The position effect of insecticide-contaminated corpses relative to the nest and its implication for Argentine ant control are discussed.

KEY WORDS *Linepithema humile*, fipronil, necrophoresis, spot treatment

Ants rank as one of the most important pest complexes in the structural pest control industry, with an estimated $1.7 billion spent annually for their control by pest management professionals (PMPs) in the United States (Curl 2005). Tramp or invasive species such as the Argentine ant, *Linepithema humile* (Mayr); *Technomyrmex albipes* (Fr. Smith); red imported fire ant, *Solenopsis invicta* Buren; crazy ant, *Paratrechina longicornis* (Latreille); Pharaoh ant, *Monomorium pharaonis* (L.); and *Tapinoma melanocephalum* (F.) thrive in disturbed habitats and are becoming more common due to increasing urbanization and global transport (Passera 1994, McGlynn 1999).

The Argentine ant is a well-established invasive pest in the United States (Hedges 1998, Vega and Rust 2001), and the most important urban pest ant species in California (Knight and Rust 1990, Gulmahamad 1997). In a survey by Field et al. (2007), Argentine ants made up 85% of the ant collections by PMPs in the San Diego area, where the Mediterranean climate and extensive irrigation in urban settings contributed to their prevalence. Even though most infestations and colonies exist outdoors (Field et al. 2007), seasonal home invasions of *L. humile* have been reported in urban settings (Knight and Rust 1990), especially during periods of cold and rainy or hot and dry weather (Gordon et al. 2001).

Control strategies for Argentine ants have primarily focused on the application of barrier sprays, granules, and baits (Rust 2001, Rust et al. 2003, Silverman and Brightwell 2008). Even with recent advances in bait technologies (Klotz et al. 2003), residual insecticide barriers are still widely used by PMPs to control the Argentine ant (Rust et al. 2003). A perimeter treatment with repellent insecticides such as pyrethroids can provide an effective barrier around the outside of a structure (Vega and Rust 2001, Rust et al. 2003). However, such treatments can trap ants within a structure, resulting in complaints and additional treatments (Rust et al. 1996, Gulmahamad 1997).

New classes of insecticides have recently been incorporated as perimeter sprays against ants, replacing organophosphate and pyrethroid insecticides. For example, barrier applications of chlorfenapyr (pyrazole) provided ≈75% reduction in Argentine ants (Suoja et al. 2000). Fipronil (phenylpyrazole) and imidacloprid (neonicotinoid) provided excellent control of several ant species, including pavement ant, *Tetramorium caespitum* (L.); *Prenolepis imparis* (Say); and odorous house ant, *Tapinoma sessile* (Say) (Scharf et al. 2004). Costa and Rust (1999) reported that soil treatments with fipronil were as effective as bifenthrin applica-

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tions against Argentine ants and *S. invicta.* Directed spot sprays of fipronil were among the most effective treatments, resulting in >90% reductions of Argentine ants around homes for at least 8 wk in southern California (Klotz et al. 2007). The success of these barrier and spot treatments has been attributed, in part, to their lack of repellency (Rust et al. 1996, Scharf et al. 2004, Klotz et al. 2007).

Ten dead ants exposed to fipronil deposit for 1 min transferred enough fipronil to nearly eliminate a 200-worker laboratory colony in 6 d, suggesting that necrophoresis was one of the important mechanism in horizontal transfer (Soeprono and Rust 2004b). Soeprono and Rust (2004b) suggested that the success of fipronil treatments in the field may be due, in part, to transfer achieved by donor–recipient physical interactions, including necrophoresis. They also suspected that the horizontal transfer facilitated by necrophoresis may likely occur when the dead ants are close to the nest. However, this hypothesis was not tested in their study because they used a single plastic container as an experimental arena, and treated dead ants were always placed close to the nest chamber. Also, little information is available concerning the magnitude and mechanism of transfer of various insecticides, including fipronil, among nestmates.

In the current study, eight insecticides, including fipronil, were tested to determine the extent of horizontal transfer among individuals in an Argentine ant colony. After determining which insecticides were effectively transferred among nestmates, we examined the importance of necrophoresis for horizontal transfer of the insecticides. The influence of the position of insecticide-contaminated corpses on the resulting mortality and its possible implication for Argentine ant control in the field are discussed.

Materials and Methods

Collecting and Maintaining Ants. Argentine ants were collected from a citrus grove on the University of California, Riverside campus. Approximately 6 ant nests were excavated from the ground and transported to a laboratory chamber where they were extracted from the soil using procedures developed by Hooper-Bui and Rust (2000). Large laboratory colonies were maintained in plastic boxes (26.5 by 30 by 10 cm, Spectrum Containers Inc., Evansville, IN) with the inner sides coated with Teflon (fluoropolymer resin, type 30, DuPont Polymers, Wilmington, DE) to prevent ants from escaping. Each colony was provided with two or three artificial nests constructed from plaster-filled petri dishes (9 cm in diameter by 1.5 cm in depth) formed with a 5-cm-diameter by 1-cm-deep cylindrical area in the center of the dish to serve as a nesting space (Soeprono and Rust 2004b, Costa et al. 2005). Each day, colonies were provisioned with fresh water, 25% (wt:vol) sucrose water, and freshly killed western drywood termites, *Incisitermes minor* (Hagen), and American cockroaches, *Periplaneta americana* (L.).

Setup of Experimental Nests. Argentine ants from the larger laboratory colony boxes were anesthetized with CO_2 and placed in an empty plastic box (26.5 by 30 by 10 cm) with the sides coated with Teflon to prevent ants from escaping. Short pieces of Tygon tubing (0.6 cm in diameter by 5.5 cm in length) each with a wet dental wick (1.5 by 2 cm) plugged into one end were placed in the box to serve as nests. As the ants recovered from anesthesia, they moved into the Tygon tubes with their brood (eggs, larvae, and pupae). Tygon tubing nests were advantageous because the ants reformed their colony quickly (within 1 h), and formed relatively similar-sized colonies with brood as well as workers and reproductives. For example, in the first experiment, the average number of ants in each experimental nest was 266.0 ± 6.7 (mean ± SEM, $n = 63$, range 169–410 ants). In the second experiment, the average number of ants in each nest was 328.6 ± 25.0 (mean ± SEM, $n = 9$, range 258–483 ants). Most of the experimental nests had at least one queen in them, whereas the average number of queens per nest was 1.7 ± 0.1 (mean ± SEM, $n = 72$). Brood size in each experimental nest ranged from 100 to 300 larvae. The Tygon tubing provided compact and effective nesting condition for the small laboratory colonies with only 8–10% mortality in the controls during the week-long experimental period. After the ants moved into a tube, it was transferred to a petri dish (9 cm in diameter by 1.5 cm in depth) with the inner sides of wall and lid coated with Teflon to prevent escape. Each dish was provisioned with 25% sucrose water in a small plastic dish. With the petri dish lid as a cover, the nest interior was maintained at ≈80% RH during the experiments.

Insecticides. The eight insecticides that we tested were as follows: acetamiprid (20 and 40 ppm; 70 WP, FMC Corp., Philadelphia, PA); bifenthrin (20 and 40 ppm; Talstar F, FMC); chlorfenapyr (20 and 40 ppm; Phantom 2 SC, BASF, Research Triangle Park, NC); two formulations of cyfluthrin (10, 20, and 40 ppm for Tempo Ultra SC, Bayer Corp., Professional Care, Kansas City, MO; 10, 20, and 40 ppm for Cy-Kick CS, Whitmire Micro-Gen Research Laboratories, Inc., St. Louis, MO); fipronil (10, 20, and 40 ppm; Termidor SC, BASF, Research Triangle Park, NC); indoxacarb (20 and 40 ppm; 1.25 SC, DuPont Agricultural Products Department, Wilmington, DE); and thiamethoxam (10, 20, and 40 ppm; 25 WG, Syngenta Crop Protection, Inc., Greensboro, NC). In total, 20 different insecticide treatments were tested. Each insecticide was prepared so that a 10-ml aqueous aliquot poured onto sand (100 g) in a Pyrex petri dish (9 cm in diameter by 2 cm in height) provided the required concentration. The sand and aqueous insecticide formulation were thoroughly stirred using a plastic spatula. Each dish was then stored at laboratory conditions (26 ± 2°C and 30–50% RH) and mixed daily until the sand was completely dry. Treated sand in the petri dishes was stored separately in a refrigerator until used. All treated sand was used within 7 d except fipronil-treated sand in the second experiment, which was used after 27 d of storage.

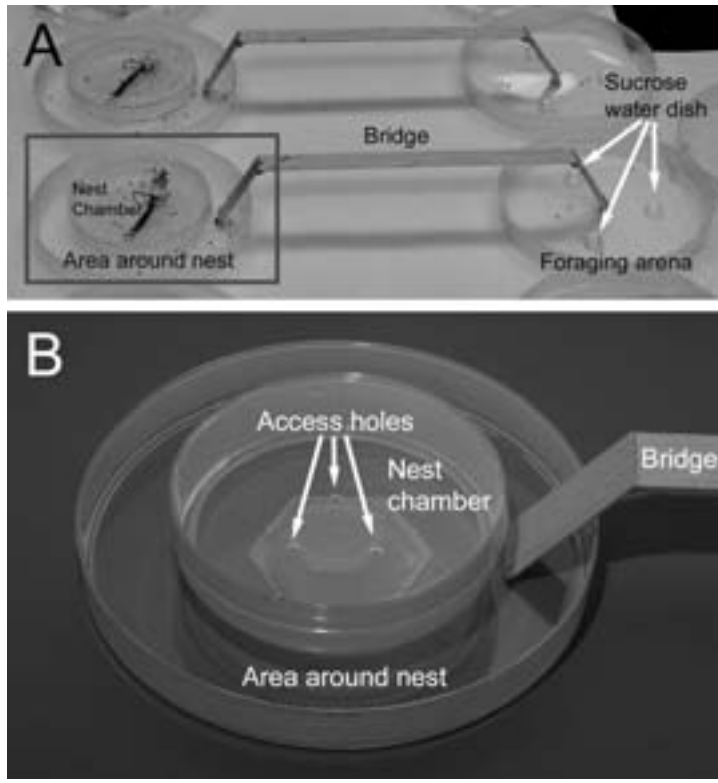

Fig. 1. (A) Three-chamber experimental arena setup. This shows three different compartments: nest chamber, area around nest, and foraging arena. To allow ants to forage, the area around the nest and the foraging arena are connected with a wooden bridge. (B) Detail of the nest chamber area with the nest tubing removed.

Exposure of Ants to Insecticides. Ants were exposed to the treated sand by using a technique developed by Soeprono and Rust (2004b). All treatments were performed by allowing ants to walk on treated sand in a large (140 by 140 by 25 mm) polystyrene weigh dish (Thermo Fisher Scientific, Waltham, MA). To confine ants to the deposits, the inner sides of the dish were coated with Teflon. The inner bottom of the dishes was coated with a thin layer of white glue (Conros Corp., Taylor, MI). Small aliquots of insecticide-treated sand were then poured into the dish so that the bottom was completely covered with sand and left to dry for 1 d. The excess sand was poured out of the dish leaving only a thin layer of treated sand adhering to the bottom. This provided a uniform substrate and permitted ants to be aspirated off the sand.

Horizontal Transfer Experiments with Treated Live Ants. To determine whether insecticides were horizontally transferred among individuals, live ants exposed to insecticides were introduced into the experimental nest, and mortality was recorded over time. First, 10 ants were aspirated from a larger laboratory colony and placed into a weigh dish containing treated or untreated (control) sand for 1 min. During this brief exposure, no physical interaction among the ants was observed. The ants were then aspirated and immediately introduced into an experimental nest. The treatment and control colonies were maintained for 7 d at 21–23°C. The number of dead ants was counted daily, and the dead ants were not removed from the petri dish. The colonies in the experimental nests were provided with fresh water by wetting the dental wick with 0.3–0.4 ml of filtered water, and 25% sucrose water in a plastic dish. This procedure was repeated three times for each of the treatments.

Horizontal Transfer Experiments with Treated Dead Ants. To determine whether the position of insecticide-contaminated dead ants in relation to the nest affected transfer, a "three-chamber experimental arena" was used (Fig. 1A). Two plastic petri dishes (14 cm in diameter by 1.5 cm in depth) with the inside walls coated with Teflon were connected with a wooden bridge (1.5 by 30 cm). One dish comprised the "area around nest," and the other was the foraging arena (Fig. 1A). In the foraging arena, three small dishes with 25% sucrose water were provided. An experimental nest attached with glue on the bottom of the area around nest served as "nest chamber." To provide ants access to the area around the nest, the experimental nest was attached on a small polystyrene weigh dish (4.5 cm in diameter) positioned upside down on the center of the area around the nest, and three access holes (3 mm in diameter) were drilled on the bottom of the experimental nest along the edge of the attached weigh dish (Fig. 1B).

Ten ants were aspirated from a larger laboratory colony and placed into a weigh dish containing treated (40 ppm only) or untreated (control) sand for 1 min. During this brief exposure, no physical interaction among the ants was observed. Then the ants were freeze-killed in a plastic container by placing them in a −60°C freezer for 10 min. To remove the extra moisture that condensed on the corpses, the dead ants were held at room temperature (21–23°C) for 10 min, and then they were placed into the experimental arena. Quickly freezing the treated ants minimized contact with other ants and ensured that each ant had similar deposits of insecticide. Howard and Tschinkel (1976) reported that the necrophoric cue in the dead *S. invicta* appeared rapidly within about an hour regardless of mode of kill (i.e., freezing or heating). To simulate the situation where ants encounter corpses near or away from the nest, insecticide-contaminated corpses were placed in the nest chamber (treatment A) or in the foraging arena (treatment B), respectively. For the control, 10 untreated freeze-killed ants were placed at similar locations in the experimental arena. The treatment and control colonies were maintained for 10 d at 21–23°C. The number of dead ants in each compartment was counted daily. Each day, the colonies were provided with fresh water by wetting the dental wick with 0.3–0.4 ml of filtered water, and 25% sucrose water in the small dishes of foraging arena. Each treatment was replicated three times.

Statistical Analyses. A repeated measures analysis of variance (ANOVA) using the SAS procedure PROC MIXED (SAS Institute 1999) was used to analyze treatment effect on cumulative mortality. Days after treatment and insecticide treatment were completely crossed as main fixed effects. Experimental replication was considered random and nested within treatment. Differences among levels of main effects were based on comparisons of corresponding least squares means. When a significant interaction was detected between the main effects, we compared each level of the between-subject factor (i.e., insecticide treatment) within each level of the within-subject factor (i.e., day after treatment) using the SLICE option of the LSMEANS statement. Normality of the data were assumed based on a normal probability plot of the residuals (SAS Institute 1999). In the first experiment, some fipronil treatments resulted in ≈10 times higher mortality than the others, and the variance values for these groups obscured the comparisons with other treatments. Thus, we first determined which insecticide treatments were significantly different from the corresponding controls by using Dunnett's test, which is relatively robust under small sample size ($n < 15$) and variance heterogeneity (Rudolph 1988). Treatments that were significantly different from the controls over time were subjected to a Fisher least significant difference (LSD) test for mean comparison. To determine when the major mortality occurred, incremental mortalities between two consecutive days were compared with controls using a repeated measures ANOVA followed by Dunnett's test (SAS Institute 1999). In the second experiment, overall counts of dead ants in the arena compartments were compared using an ANOVA followed by Fisher LSD (Analytical Software 2000).

Results

Horizontal Transfer Experiments with Treated Live Ants. The cumulative number of ants that died after the insecticide treatments increased significantly over time (Repeated measures ANOVA, $F = 428.88$, df = 7, 294; $P < 0.0001$). A significant interaction occurred between day and treatment indicating that treatments were significantly different in terms of their mortality change throughout the experimental period (repeated measures ANOVA, $F = 40.88$; df = 140, 294; $P < 0.0001$).

Ants exposed to fipronil deposits (20 and 40 ppm) transferred the insecticide to other individuals, resulting in the highest mortality. The treatments with 20 and 40 ppm fipronil killed significantly more ants than did the controls throughout the entire experimental period except at day 1 posttreatment with 40 ppm (LSMEANS: $P < 0.001$) (Table 1). At 10 ppm, fipronil was not transferred sufficiently to be lethal to nestmates, and the colony mortality was not significantly different from the controls (Table 1). At the end of the trial (day 7), the average number of dead ants was significantly higher than the controls (18.0 ± 2.0) for 20 ppm (103.0 ± 16.8; LSMEANS: $t = 18.02$; df = 294; $P < 0.001$), and 40 ppm (139.0 ± 22.3; LSMEANS: $t = 25.65$; df = 294; $P < 0.001$), but not for 10 ppm fipronil (29.3 ± 10.2; LSMEANS: $t = 2.40$; df = 294; $P = 0.52$) (means ± SD) (Table 1). From day 3 posttreatment to the end of the study, the cumulative number of dead ants was significantly higher in the 40 ppm treatment than it was in the 20 ppm treatment (LSMEANS: day 3, $t = -2.40$, df = 294, $P = 0.02$; day 4, $t = -5.02$, df = 294, $P < 0.0001$; day 5, $t = -6.36$, df = 294, $P < 0.0001$; day 6, $t = -7.35$, df = 294, $P < 0.0001$; day 7, $t = -7.63$, df = 294, $P < 0.0001$) (Table 1).

The other insecticides were not transferred in amounts that were lethal to nestmates (Table 1). Although some treatments had some higher initial mortality (e.g., bifenthrin 20 and 40 ppm; Tempo Ultra 20 ppm), or lower overall mortality (e.g., acetamiprid 40 ppm; Tempo Ultra 10 ppm; Cy-Kick 10 ppm), none of them were statistically different from the control (LSMEANS: $P > 0.05$). At the end of the trial, average counts of dead ants in all of the other insecticide treatments besides fipronil ranged from 3.7 ± 0.6 to 24.7 ± 9.5 (means ± SD). The average number of dead ants in the controls was 18.0 ± 2.0 (mean ± SD) during the same time period (Table 1).

The incremental mortality between two consecutive days indicated that higher mortality occurred between days 0 and 4 posttreatment than between days 4 and 7 posttreatment in the two fipronil treatments (20 and 40 ppm) (Fig. 2). The largest incremental change occurred between days 1 and 2 posttreatment with 40 ppm (Fig. 2). Both the 20 and 40 ppm fipronil treatments provided significant transfer and kill of workers for 3 d posttreatment. However,

Table 1. Cumulative number of dead ants in the experimental nest after the addition of 10 live ants previously exposed to various insecticide treatments

Treatment[a]	Time after treatment (d)						
	1	2	3	4	5	6	7
Control	3.7 ± 2.1a	8.0 ± 2.0a	11.3 ± 3.2a	15.3 ± 2.5a	17.3 ± 1.2a	17.7 ± 1.5a	18.0 ± 2.0a
Fipronil 10 ppm	12.0 ± 4.4a	16.0 ± 5.2a	19.0 ± 8.7a	23.7 ± 9.6a	25.3 ± 10.4a	27.3 ± 8.7a	29.3 ± 10.2a
Fipronil 20 ppm	25.7 ± 4.5bA	49.7 ± 11.5bA	74.0 ± 10.5bA	82.3 ± 9.6bA	87.3 ± 14.2bA	93.0 ± 17.7bA	103.0 ± 16.8bA
Fipronil 40 ppm	19.7 ± 0.6aA	56.0 ± 6.2bA	85.3 ± 7.6bB	106.0 ± 18.2bB	117.3 ± 17.6bB	127.7 ± 16.5bB	139.0 ± 22.3bB
Acetamiprid 20 ppm	1.3 ± 1.2a	5.7 ± 1.2a	11.0 ± 1.3a	13.3 ± 0.6a	14.3 ± 0.6a	14.3 ± 0.6a	14.3 ± 0.6a
Acetamiprid 40 ppm	0.3 ± 0.6a	1.3 ± 0.6a	2.3 ± 0.6a	2.3 ± 0.6a	2.7 ± 0.6a	3.3 ± 1.2a	3.7 ± 0.6a
Bifenthrin 20 ppm	16.0 ± 2.6a	18.0 ± 5.3a	20.0 ± 5.3a	21.3 ± 5.1a	21.7 ± 5.0a	22.3 ± 4.2a	22.3 ± 4.2a
Bifenthrin 40 ppm	15.3 ± 6.8a	16.3 ± 5.8a	17.0 ± 7.8a	18.0 ± 6.9a	19.0 ± 6.2a	19.3 ± 6.1a	20.3 ± 6.0a
Chlorfenapyr 20 ppm	5.0 ± 2.0a	10.7 ± 4.0a	12.3 ± 3.8a	13.7 ± 5.1a	16.0 ± 4.4a	16.0 ± 4.4a	16.7 ± 3.2a
Chlorfenapyr 40 ppm	4.3 ± 1.5a	6.3 ± 3.2a	7.7 ± 2.1a	9.3 ± 1.2a	10.0 ± 1.0a	10.0 ± 1.0a	10.3 ± 1.2a
Cyfluthrin (T) 10 ppm	5.3 ± 4.2a	5.7 ± 4.6a	6.7 ± 3.8a	7.0 ± 4.4a	8.0 ± 4.0a	8.3 ± 3.5a	8.3 ± 3.5a
Cyfluthrin (T) 20 ppm	13.7 ± 1.5a	15.0 ± 2.6a	17.0 ± 2.6a	17.7 ± 2.5a	19.0 ± 3.6a	19.3 ± 4.0a	21.7 ± 2.3a
Cyfluthrin (T) 40 ppm	70.0 ± 5.0a	8.3 ± 6.8a	8.3 ± 5.5a	9.0 ± 6.6a	9.3 ± 6.1a	10.3 ± 6.1a	11.3 ± 5.1a
Cyfluthrin (C) 10 ppm	5.3 ± 2.5a	5.7 ± 2.5a	6.7 ± 3.2a	7.3 ± 3.8a	8.0 ± 4.4a	8.0 ± 4.4a	9.0 ± 2.6a
Cyfluthrin (C) 20 ppm	7.7 ± 1.2a	8.3 ± 1.5a	9.3 ± 1.5a	9.3 ± 1.5a	8.3 ± 0.6a	8.7 ± 0.6a	10.7 ± 0.6a
Cyfluthrin (C) 40 ppm	9.3 ± 1.5a	10.7 ± 1.5a	11.0 ± 1.0a	11.7 ± 1.2a	12.0 ± 1.0a	12.3 ± 0.6a	12.3 ± 0.6a
Indoxacarb 20 ppm	10.0 ± 5.0a	14.7 ± 5.8a	17.0 ± 5.3a	20.7 ± 6.8a	22.7 ± 8.4a	23.7 ± 8.5a	24.7 ± 9.5a
Indoxacarb 40 ppm	7.0 ± 3.6a	9.0 ± 2.6a	10.3 ± 3.1a	10.7 ± 3.2a	12.3 ± 2.5a	13.0 ± 3.0a	14.3 ± 5.2a
Thiamethoxam 20 ppm	3.0 ± 1.0a	5.7 ± 2.9a	7.3 ± 4.0a	9.0 ± 6.1a	9.7 ± 7.2a	10.7 ± 7.4a	11.0 ± 7.0a
Thiamethoxam 40 ppm	5.3 ± 1.5a	10.7 ± 3.8a	10.7 ± 1.2a	12.0 ± 1.0a	13.0 ± 1.7a	14.3 ± 2.3a	14.3 ± 2.3a

Within each column, values (means ± SD) ($n = 3$) marked with a lowercase "a" are not significantly different from corresponding control ($\alpha = 0.05$; Dunnett's test). For fipronil 20 and 40 ppm treatments, values (mean ± SD) ($n = 3$) followed by the same uppercase letter within each column are not significantly different ($\alpha = 0.05$; Fisher LSD).

[a] For cyfluthrin, T is Tempo Ultra, C is Cy-Kick CS.

only at 40 ppm did fipronil continue to be active for 7 d posttreatment (Fig. 2).

Horizontal Transfer Experiments with Treated Dead Ants (40 ppm Fipronil). The cumulative number of dead ants significantly increased over time (repeated measures ANOVA: $F = 252.07$; df = 10, 60; $P < 0.0001$). A significant interaction occurred between day and treatment indicating that the mortality over time was not equal for the different treatments (repeated measures ANOVA: $F = 43.15$; df = 20, 60; $P < 0.0001$) (Fig. 3).

The location of the treated dead ants significantly affected mortality. From day 6 posttreatment to the end of the study, the cumulative number of dead ants was significantly higher in the treatment where the treated dead ants were placed in the nest (treatment A) than it was in the treatment where they were placed in the foraging arena (treatment B) (LSMEANS: day 6, $t = 2.39$, df = 60, $P = 0.02$; day 7, $t = 2.07$, df = 60, $P = 0.04$; day 8, $t = 2.52$, df = 60, $P = 0.01$; day 9, $t = 2.74$, df = 60, $P = 0.01$; day 10, $t = 2.90$, df = 60, $P = 0.01$) (Fig. 3). At the end of the trial,

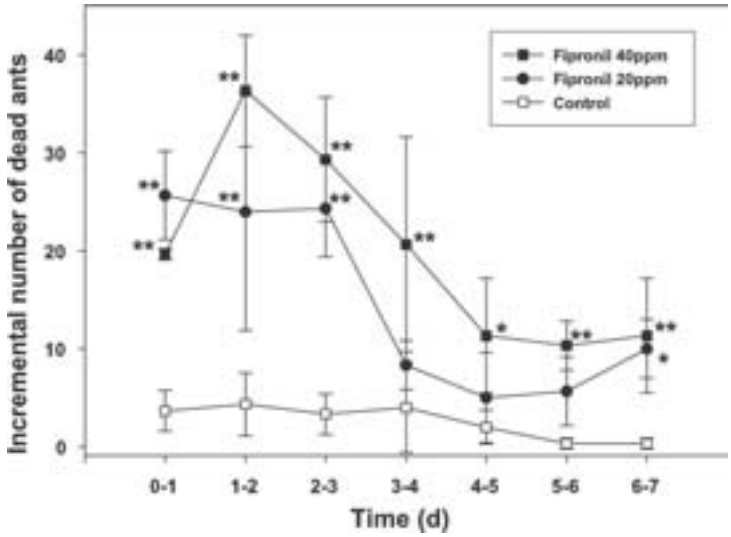

Fig. 2. Incremental number of dead ants between two consecutive days in two fipronil treatments (20 and 40 ppm) and control. Values represent means ± SD. An asterisk (*) represents significant differences between the treatment group and corresponding controls (**, $P < 0.0001$ and *, $P < 0.01$; Dunnett's test).

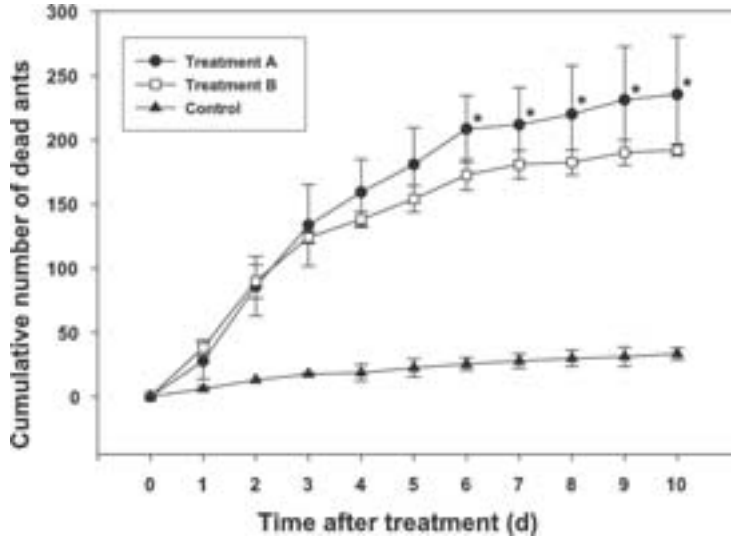

Fig. 3. Cumulative number of dead ants in the three-chamber experimental arena after the addition of 10 freeze-killed ants which were previously exposed to fipronil on sand (40 ppm). The insecticide-treated dead ants were initially placed in the nest chamber (treatment A) or in the foraging arena (treatment B). Values represent means ± SD. An asterisk (*) indicates that treatments A and B differed from one another on that day ($P < 0.05$; Fisher LSD).

average counts of dead ants were 235.3 ± 45.1 (treatment A), 192.0 ± 4.6 (treatment B), and 33.3 ± 4.5 (control) (means ± SD) (Fig. 3).

The majority of ants was found dead in the vicinity of their nest. At the end of the trial, the number of corpses recovered from the nest area (nest chamber + area around nest) consisted of 89.7, 87.0, and 99.0% of the total dead ants in treatments A, B, and control, respectively. A significant interaction between location and treatment indicated that the initial position of treated ants significantly affected the resulting mortality in each compartment ($F = 26.20$; df = 2, 12; $P < 0.0001$). The numbers of dead ants in the nest area were significantly different among treatments A, B, and control, whereas the foraging arenas had similar numbers of dead ants in them (Fig. 4).

Discussion

A slow-acting, nonrepellent insecticide such as fipronil allows social insects, including ants, time to continue foraging on a treated surface and recruit

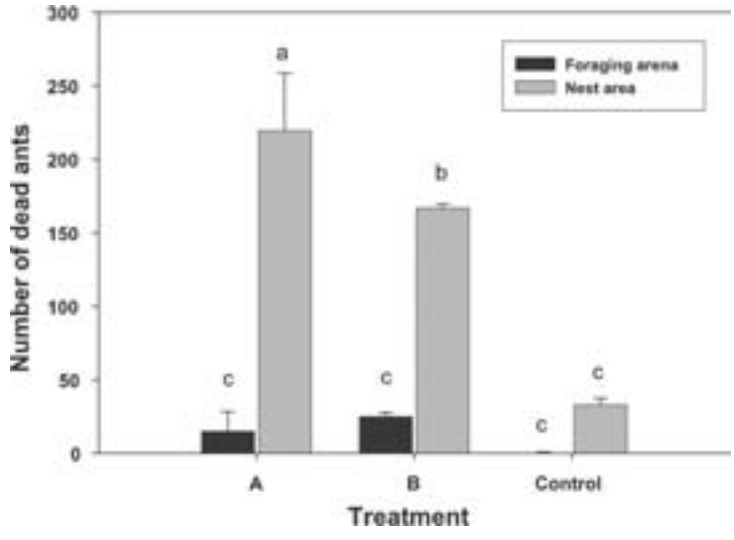

Fig. 4. Number of dead ants recovered in the foraging arena and the nest area. The insecticide-treated dead ants were initially placed in the nest chamber (treatment A) or in the foraging arena (treatment B). At the end of the trial, ant corpses in the foraging arena and the nest area (nest chamber + area around nest) were counted separately. Values represent means ± SD. Means with the different letter are significantly different ($P < 0.05$; Fisher LSD).

nestmates, resulting in a greater proportion of the colony being exposed to the toxicant (Soeprono and Rust 2004a). Based on their termite studies, Thorne and Breisch (2001) and Saran and Rust (2007) suggested that fast-acting, repellent insecticides would result in fewer insects being exposed and killed. The combination of delayed toxicity and nonrepellency might also facilitate horizontal transfer of the insecticide among social or aggregating insect pests, and thus provide control away from the treated area (Buczkowski and Schal 2001, Ibrahim et al. 2003, Shelton and Grace 2003).

Soils treated with bifenthrin, cyfluthrin, and fipronil ranging from 1.4 to 19.2 ppm were tested by Soeprono and Rust (2004a,b) for their effects on the recruitment behavior of Argentine ants and subsequent kill by horizontal transfer. In the current study, we included additional insecticides which are being used for ant control, and also examined greater concentration range (10, 20, and 40 ppm) to determine whether transfer effects were dose dependent. According to Soeprono and Rust (2004b), relatively high mortality in the controls (18–35%) during a week-long experimental period prevented a more definitive interpretation of the results in their transfer experiments. Our bioassay had relatively low control mortality over a similar period of 7–10 d (i.e., 8–10 and 6–11% for the first and second experiments, respectively). This was probably due to improvements in the experimental setup and design. Because worker ants simultaneously formed several small colonies by aggregating brood into the tubing pieces, we could prepare many small experimental colonies in a relatively short time, while avoiding any risk of disturbing or accidentally killing ants. Even though the total number of ants in the tubing nest was variable, workers formed reasonably uniform colonies.

The results of the first experiment with treated live ants indicated that only fipronil was transferred from exposed to unexposed ants at a sufficient level to kill nestmates in a small colony. Recipients picked up lethal doses because fipronil is nonrepellent and exhibits extremely high contact toxicity to insects (Buczkowski and Schal 2001). However, exposure to concentrations >10 ppm was necessary for transfer to occur. The small percentage of exposed ants in our test colonies (an average of 3.9%), and the relatively brief exposure period (1 min) might have influenced these results. Shelton and Grace (2003) demonstrated that exposing more termites might allow for insecticide transfer to occur at lower concentrations. A longer exposure time allowed termites to pick up higher doses of insecticide from a treated substrate, but this also decreased the time required to kill them (Saran and Rust 2007). An increase in speed-of-kill decreased the distance that termites tunneled.

Among the fipronil treatments with higher concentrations (i.e., 20 or 40 ppm), recipient mortality was dose dependent, with higher mortality in the 40 ppm treatment. Dose dependency of fipronil transfer has been reported in other insects, including termites (Shelton and Grace 2003) and cockroaches (Buczkowski and Schal 2001). Saran and Rust (2007) reported that subterranean termites, which have been exposed to higher concentrations of fipronil had greater amounts of insecticide on their bodies, resulting in higher levels of transfer to recipients.

Soeprono and Rust (2004b) stated that the persistence of fipronil horizontal transfer within an ant colony is not well understood. In our experiments, most of the transfer and subsequent mortality took place within 4 d. After day 4, there was a negligible kill compared with before day 3. In similar brief exposure tests, Soeprono and Rust (2004a) found that most Argentine ants (90%) exposed to 19.2 ppm fipronil were killed within 120 min. Thus, we assumed that all the donors in the 20 or 40 ppm fipronil treatments would die by day 1 posttreatment. Saran and Rust (2007) reported that the maximum transfer of fipronil took place within 24 h after introducing donors into a subterranean termite colony. Our study clearly indicated that the initial amount of fipronil transferred affected the persistence of the transfer effect because the 40 ppm fipronil remained effective longer than did the 20 ppm fipronil.

The results of the second experiment with treated dead ants clearly demonstrated that a significant amount of horizontal transfer occurred through contact with insecticide-contaminated corpses. The chance of accidental contact between unexposed ants and the dead donors was minimized (but not prevented) by placing the donors in a separate foraging arena. However, the dead ants were frequently moved about by other workers. Thus, the mechanism of horizontal transfer can be mostly attributed to necrophoresis (i.e., carrying of dead nestmates to refuse piles), one of the most stereotypic social behaviors of ants (Wilson et al. 1958). The significance of necrophoresis in insecticide transfer is highly variable among different social insects. In some termites, for example, dead or decomposing nestmates killed by a contact insecticide are frequently covered with soil and isolated from live termites (Su 2005). In this situation, horizontal transfer of insecticide by necrophoresis may be negligible.

More ants picked up lethal doses of fipronil when the dead exposed ants were placed in the nest. The crowded nesting conditions might be partially responsible for more frequent contacts with the insecticide-contaminated corpses or other substrate upon which dead ants were placed. In addition, more ants in the nest might be motivated to examine and carry dead ants if a larger percentage of them are engaged in nest maintenance activities (Gordon 1983). Soeprono and Rust (2004b) hypothesized that if ants were exposed to a treated area far from their nest, they might die before returning to it, resulting in minimal horizontal transfer. The closer a treatment is to the nest, the greater the likelihood that a toxicant would be transferred throughout the colony (Soeprono and Rust 2004b). We suspect that the efficacy of target applications of fipronil may be due, in part, to this transfer effect. In one field study, a fipronil spot treatment at 60–75 ppm (mass active ingredient/mass dry soil) on

active ant trails reduced Argentine ant activity by 90% around houses with only 25% the amount of insecticide as was used in a conventional perimeter treatment (Klotz et al. 2007).

In our experiments, most of the dead ants (≈90%) were found near the nest even when the dead donor ants were originally placed in the foraging arena. We could not determine whether the ants had picked up a lethal dose of fipronil and became immobilized while they were near the nest, or if they died away from the nest and were then moved closer to the nest. However, Argentine ants typically move corpses between experimental arena chambers, frequently transporting them to an existing refuse pile in the vicinity of the nest (D.-H.C., unpublished data). Because natural refuse piles of observed Argentine ant nests were located within 3–5 m from the nest entrances (D.-H.C., unpublished data), most of the dead ants will be found within this area after fipronil treatment. Faye (1996) reported high necrophoric activity and active formation of dead pile in mound vicinity when insecticidal rotenone was applied in *S. invicta* nest as a drench formulation. Our study suggests that spot treatments of fipronil would be more effective when they are applied closer to the nest, because more fipronil-contaminated corpses will accumulate in this area, maximizing the possibility of horizontal transfer by physical contact.

Of the eight insecticides we tested at 10–40 ppm, only fipronil was horizontally transferred among nestmates to provide significant secondary kill. In addition to its high contact toxicity and nonrepellency, intermediate affinity for lipids (e.g., wax layer of insect cuticle) may contribute to its horizontal transfer. For example, octanol-water partition coefficient (log K_{OW}) value of fipronil is 4.0, whereas log K_{OW} values of other active ingredients were lower (acetamiprid, 0.8; thiamethoxam, −0.1) or higher (bifenthrin, 6.0; chlorfenapyr, 5.0; cyfluthrin, 5.9; indoxacarb, 4.7) than that of fipronil. The type of substrate, concentration of insecticide, exposure time of donor, and elapsed time after exposure would greatly affect the efficacy of insecticide transfer (Rust and Saran 2006). Along with other laboratory and field studies (Soeprono and Rust 2004a,b; Klotz et al. 2007), our studies suggest that spot treatment of 20–40 ppm fipronil near the nest or on trails might enhance control of Argentine ants, thereby eliminating the need of extensive barrier treatments.

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