# Effect of Horizontal Transfer of Barrier Insecticides to Control Argentine Ants (Hymenoptera: Formicidae)

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ABSTRACT Horizontal transfer of three contact insecticides, bifenthrin,  $\beta$ -cyfluthrin, and fipronil, was tested in laboratory colonies. Donor ants were exposed for 1 min to insecticide-treated sand substrates and placed with unexposed ant colonies at two different temperatures. Mortality was monitored to compare the ability of donors to transfer lethal doses of these insecticides to untreated individuals. Treated donor insects, live or dead, were added into colonies to determine the importance of donor behavior on lethal transfer. Fipronil was readily transferable between individuals, resulting in high mortality rates. Bifenthrin and  $\beta$ -cyfluthrin were less transferable, exhibiting moderate-to-low mortality rates similar to the controls. Greater mortality occurred at 27–29°C than at 21–23°C for bifenthrin, but not the other treatments or controls. Colony mortality did not significantly increase when adding live donors, suggesting that necrophoresis was probably an important donor behavior in addition to grooming and trophallaxis on horizontal transfer.

KEY WORDS Argentine ant, horizontal transfer, fipronil, bifenthrin, cyfluthrin

HORIZONTAL TRANSFER OF INSECTICIDES is the movement of an active ingredient from one individual to another individual by physical contact. In a social, semisocial, or aggregating group of insects, this may happen directly through member-to-member contact or indirectly by contact with a substrate that has been indirectly contaminated. Trophallxis might facilitate horizontal transfer in these groups when orally transferring nutrients and semiochemicals between individuals in social insects such as ants, termites, and some wasps and bees (Hölldobler and Wilson 1990) and is the primary means of transferring toxicants in baits against ants and termites (Stringer et al. 1964, Su et al. 1991). Past studies dealing with horizontal transfer have predominantly focused on the control of cockroaches (Kopanic and Schal 1997, 1999; Buczkowski and Schal 2001), mosquitoes (Chism and Apperson 2003), termites (Randal and Doody 1934, Myles 1994, Myles et al. 1994, Valles and Woodson 2002, Haagsma 2003, Shelton and Grace 2003), and fleas (Jacobs et al. 2000, Metzger and Rust 2002).

Active ingredients may be horizontally transferred among individuals by passive translocation of insecticides. Contact insecticides that are encountered in the environment may be brought back to aggregations or nests on body parts. For example, Shelton and Grace (2003) found that with termites, imidacloprid and fipronil could be transferred if workers were allowed to contact termites that had been exposed to 100 ppm deposits of these active ingredients on sand. However, exposure to 1 ppm did not consistently lead to a lethal transfer of the active ingredient even when donors were exposed to the treated sand for up to 24 h. In another study, imidacloprid was transferred (8.4-21.1% transfer efficiency) by contact between exposed and nonexposed termites with sealed mouthparts after exposing donors for 2 h on sand-treated substrates (500 ppm) (Haagsma 2003). Valles and Woodson (2002) showed that *Coptotermes formosanus* Shiraki workers were able to transfer a lethal dose of various termiticides to untreated workers in small containers. This transference amplified the effect of chlorpyrifos, cypermethrin, and chlordane by 1.4-, 1.5-, and 1.3-fold, respectively.

Using the insect growth regulator (IGR) pyriproxyfen, Chism and Apperson (2003) were able to inhibit adult emergence from artificial oviposition sites in Aedes albopictus (Skuse) and Ochlerotatus triseriatus (Say). In this system, gravid Ae. albopictus and Oc. triseriatus females were exposed to treated paper surfaces containing 0.3 and 0.4 and 0.2 mg/cm<sup>2</sup>, respectively, and transferred the IGR to oviposition containers. Treatment of five females resulted in 70% inhibition of emergence in Ae. albopictus, whereas the same exposure resulted in 59-73% inhibition in Oc. triseriatus. Similarly, adult cat fleas failed to develop and emerge from blankets that had been used regularly by treated domestic cats (Jacobs et al. 2000). Treating the cats with a topical formulation of imidacloprid (≥10 mg/kg b.wt.) resulted in enough transference of the active ingredient to the larval flea environment to reduce emergence by 100, 84, and 60% at week 1, 2, and 3, respectively. Metzger and Rust (2002) investigated the amount of fipronil and imidi-

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cloprid transferred from treated California ground squirrels, *Spermophilus beecheyi*, to their nesting materials, but found it negligible.

Active ingredients also may be actively transferred among individuals through characteristic social behaviors such as mutual grooming, trophallaxis, coprophagy, necrophagy, and necrophoresis. Randal and Doody (1934) used slow-acting poison dusts such as Paris green, barium fluosilicate, and arsenical smelter dust to treat drywood, dampwood, and subterranean termites in the laboratory and field. They found that injections of small quantities of these dusts into foraging tunnels and nests by using dust guns were effective in controlling western drywood termites, Incisitermes minor (Hagen), in the field. The nymphs transferred active ingredient to other nymphs by tracking the fine dust throughout their network of tunnels and by mutual grooming. To exploit mutual grooming, Myles (1994) described a trap-treat-release technique in which eastern subterranean termites, Reticulitermes flavipes Kollar, were collected from residential properties, treated with a resin-based coating containing sulfluramid, and released back into their original colonies. The donor termites delivered active ingredient into their respective colonies through mutual grooming and propagated the spread of active ingredient by trophallaxis.

Kopanic and Schal (1999) showed that sedentary first and second instars of German cockroach, *Blattella germanica* L., received lethal doses of hydamethylnon by consuming adult excreta (coprophagy) at aggregation sites. The use of a slow-acting insecticide, such as hydramethylnon, was important because most of the active ingredient was excreted 12-h postconsumption. Fipronil also was carried back to harborages by German cockroaches and transferred to other individuals in the aggregation (Buczkowski and Schal 2001). The method by which the insecticide was delivered to the donor—topical application, surface contact, or ingestion of fipronil—affected the degree of transference and secondary mortality.

Smith and Lockwood (2003) reported that there was a 100% lethal transfer from grasshopper cadavers fed fipronil-sprayed foliage (250 times the label rate of 4 g AI/ha) to conspecific cannibal grasshoppers for three trophic passages. At 25 and 1 times the label rate, fipronil showed lethal transfer for one passage. It was suggested that the transfer of fipronil through necrophagy may increase the efficacy of control programs aimed at grasshoppers and locusts.

In the current study, we tested three insecticides registered as barrier sprays for ant control with regard to their ability to be horizontally transferred between members of the colony. In the first experiment, 10 live workers briefly exposed to bifenthrin,  $\beta$ -cyfluthrin, or fipronil deposits were placed in laboratory colonies at two different temperature ranges to determine whether horizontal transfer occurred. In the second experiment, 10 dead workers that had been exposed to these same insecticides were freeze-killed and added to colony boxes to determine whether donor behaviors such as necrophoresis were important for horizontal transfer and subsequent mortality and to eliminate behaviors such as grooming and trophallaxis. The level of lethal transfer of these compounds to other members of the colony by treatment, temperature, and donor behavior was compared. The implications of horizontal transfer for controlling Argentine ants are discussed.

# **Materials and Methods**

Collecting and Maintaining the Ants. The ants were collected from the biological control groves on the University of California, Riverside, campus. Ant nests were dug up from the field and transported to a laboratory chamber in plastic 18.9-liter buckets coated with fluoropolymer resin (product type 30, DuPont, Washington, WV). The ants and dirt were spread thinly within a large wooden collection box with fluoropolymer resin-painted sides to prevent escape. Plaster of Paris disks soaked in water were stacked in the center of the collection box. As the soil dried, the ants moved into the moist plaster disks and could be tapped off the disks and into their final colony boxes.

Insecticides Used. The chemicals tested in our study were bifenthrin (Talstar F 7.9% [AI], FMC Corp., Philadelphia, PA), cyfluthrin (Tempo Ultra SC 11.8% [AI], Bayer Corp., Kansas City, MO), and fipronil (Termidor SC 9.1% [AI], Aventis Environmental Science, Montvale, NJ). Bifenthrin and cyfluthrin affect the sodium ion channels of neuron terminals producing sporadic neurotransmission followed by paralysis and death (Fecko 1999). Fipronil is a neurotoxin that blocks the voltage-gated chloride ion channels of the  $\gamma$ -aminobutyric acid receptor. This interferes with normal inhibition, resulting in uncontrolled neuronal activity, paralysis, and death (Connelly 2001).

Insecticide Preparations. Insecticides were prepared as follows: (1) 0.7 ml of Talstar F; (2) 0.38 ml of Tempo SC; or 3) 0.82 ml of Termidor SC was added to 750 ml of deionized water and shaken vigorously. Control sand was treated with 750 ml of deionized water. Each insecticide preparation (750 ml) was then poured onto sand (4.2 kg) in porcelain trays and mixed thoroughly by hand by using a steel spatula. Each tray was then stored at laboratory conditions ( $26 \pm 2^{\circ}C$ , 30-50% RH) and mixed daily until the sand was completely dry. This resulted in sands being treated at label recommended rates (mass active ingredient/ mass sand) for ant control: 13.7 ppm bifenthrin, 10.8 ppm β-cyfluthrin, and 18.8 ppm fipronil. Treated sands were stored separately in porcelain trays at laboratory conditions until used. All treated sands were used within 1 wk of their preparation.

**Exposure Apparatus.** The sides of large (140 by 140 by 25-mm) polystyrene weighing dishes (Fisher, Pittsburgh, PA) were coated with fluoropolymer resin to prevent ants from escaping. The inner bottoms of the polystyrene dishes were coated with a thin layer of white glue (Conros Corp., Taylor, MI). Small aliquots of insecticide-treated sand were then poured into the polystyrene dishes so that the bottom of the dish was completely covered with sand and left to dry 1 d. The

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Insecticide	Temp (°C)	n	Slope ± SE	LT <sub>50</sub> (d) (CI 95%)	LT <sub>95</sub> (d) (CI 95%)
Fipronil	21-23	230	$0.123 \pm 0.013$	6.4	19.8
Bifenthrin	21-23	230	$0.035 \pm 0.023$	63.7	110.1
β-Cyfluthrin	21-23	230	$0.049 \pm 0.015$	34.7	68.6
Fipronil	27-29	270	$0.207 \pm 0.017$	6.0	13.9
Bifenthrin	27-29	270	$0.181 \pm 0.022$	10.5 (7.36-26.91)	19.5 (13.05-61.73)
β-Cvfluthrin	27-29	270	$0.060 \pm 0.042$	44.3	71.5

Table 1. Mortality slopes and lethal time (LT) values in Argentine ant colonies treated with 10 live workers previously exposed to various insecticide deposits

Mortality slopes and LT values generated using probit analysis of correlated data (Throne et al. 1995).

## Results

Horizontal Transfer with Live Ants. At 21–23°C, the pretreatment mortality for all the laboratory colonies was not significantly different. An average of  $5.8 \pm 2.0$  ants died and were replaced per day before the tests. Table 1 shows that the projected time required to produce 50 and 95% mortality in fipronil-treated colonies (6.4 and 19.8 d) was significantly less than in bifenthrin (63.7 and 110.1 d) and  $\beta$ -cyfluthrin (34.8 and 68.6 d) colonies. Fipronil had the highest mortality rate (slope of 0.123).  $\beta$ -cyfluthrin (slope of 0.049) had a similar mortality slope to bifenthrin (slope of 0.035) (Table 1). The number of ants that had died by day 6 posttreatment in fipronil-treated colonies was significantly higher than in bifenthrin,  $\beta$ -cyfluthrin, and the controls ( $F_{3,16} = 11.39$ ; P < 0.001; Fig. 1). At the end

of the experiment, 97% (fipronil), 46% ( $\beta$ -cyfluthrin), and 36% (bifenthrin) of the workers were killed. In the controls, 28% of the ants died within the same time period.

At 27–29°C, pretreatment mortality for all colonies was not significantly different from one another. An average of 5.7  $\pm$  2.2 ants died and were replaced per day in the pretreatment boxes. The time required to produce 50 and 95% kill in the fipronil colonies (6.0 and 13.9 d) was significantly less than in bifenthrin (10.5 and 26.9 d), which was significantly less than  $\beta$ -cyfluthrin (44.3 and 73.5 d; Table 1). At this temperature, the slope of bifenthrin (0.181) was not significantly different than that of fipronil (0.207) or  $\beta$ -cyfluthrin (0.073). The slope of fipronil was significantly steeper than that of  $\beta$ -cyfluthrin (Table 1). The





β-Cyfluthrin

Insecticide	Temp (°C)	n	Slope ± SE	LT <sub>50</sub> (d) (CI 95%)	LT <sub>95</sub> (d) (CI 95%)			
Fipronil	21-23	361	$0.348 \pm 0.023$	3.5 (0.52-7.23)	8.2 (5.467-26.26)			
Bifenthrin	21-23	241	$0.053 \pm 0.034$	47.4	78.6			

 $0.081 \pm 0.052$ 

Table 2. Mortality slopes and lethal time (LT) values in Argentine ant colonies treated with 10 dead workers previously exposed to various insecticide deposits

Mortality slopes and LT values generated using probit analysis of correlated data (Throne et al. 1995).

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number of ants killed by day 6 posttreatment was as follows: fipronil > bifenthrin >  $\beta$ -cyfluthrin = control ( $F_{3,16} = 16.33$ ; P < 0.001; Fig. 1). At the end of the trial, 99% (fipronil), 67% (bifenthrin), and 34% ( $\beta$ -cyfluthrin) of the workers were killed. Mortality in the controls was 35% during the same time period.

21-23

The time required to kill 50 and 95% of the ants was significantly longer at 21–23°C than at 27–29°C overall  $(F_{1,32} = 7.39; P = 0.011)$ . However, only bifenthrin had both a significantly steeper mortality rate (slopes of 0.035 at 21–23°C versus 0.181 at 27–29°C; Table 1) and mortality at day 6 posttreatment ( $t_8 = 4.42; P = 0.002;$  Fig. 1). Fipronil showed a steeper mortality rate at higher temperatures (0.123 at 21–23°C versus 0.207 at 27–29°C; Table 1), but mortality at day 6 posttreatment was not significantly higher ( $t_8 = 1.34; P = 0.403;$  Fig. 1).  $\beta$ -Cyfluthrin and control mortality rates and mortality were not significantly affected by temperature (Fig. 1).

Horizontal Transfer with Dead Ants. Pretreatment mortality slopes generated by probit analyses were not significantly different during the acclimation period. The time required to kill 50 and 95% of the ants exposed to fipronil (3.5 and 8.2 d, respectively) was significantly less than for colonies exposed to bifenthrin (47.4 and 78.6 d, respectively) and  $\beta$ -cyfluthrin (34.6 and 54.9 d, respectively; Table 1). Fipronil mortality rate (slope of 0.348) was significantly different from mortality rates of bifenthrin (slope of 0.053) and  $\beta$ -cyfluthrin (slope of 0.081), which were not significantly different from one another (Table 1).

The overall ANOVA by donor status indicated that mortality in colonies day 6 posttreatment exposed to live versus dead donors was not significantly different



34.6

## Discussion

The horizontal transfer of insecticides can be achieved in several different ways. Active ingredient may be transferred passively from exposed ants to resources such as water, food supplies, or substrates. Active ingredient may be transferred by contact such as ant-to-ant tarsal or antennal contact as workers pass on a recruitment trail. Ants exposed to a barrier treatment may die in or near the nest and the active ingredient may be transferred when dead ants are carried to refuse piles by other workers (necrophoresis; Figs. 2 and 3). Other social activities such as trophallaxis or cooperative grooming also may transfer active ingredient between ants.

Fipronil achieved the highest mortality due to horizontal transfer with mortality occurring daily at both temperatures. Both mortality rates and mortality by day 6 posttreatment were significantly greater than bifenthrin,  $\beta$ -cyfluthrin, and the controls (Fig. 1). On day 6 posttreatment, there were only a few live ants in fipronil-treated colony boxes, and the trials were terminated. Bifenthrin produced significantly greater



Fig. 2. Worker carrying dead ant killed by exposure to fipronil-treated sand.



Fig. 3. Graveyard of ants outside killed by exposure to donor ants that were released into the colony box.

54.9

mortality than controls at 27–29°C (Fig. 1).  $\beta$ -Cyfluthrin never achieved mortality rates or mortality at day 6 posttreatment that were significantly greater than the controls (Fig. 1).

Temperature had a significant effect on bifenthrin. At 27-29°C, bifenthrin had both a significantly higher mortality rate and number of dead ants day 6 posttreatment than it did at 21-23°C (Table 1; Fig. 1). The significance of temperature to horizontal transfer of fipronil is questionable. Whereas probit analysis of correlated data showed a steeper slope at the higher temperature (Table 1), the number of ants that died was not significantly different at day 6 posttreatment (Fig. 1). Within the time period of this study, temperature did not significantly affect the level of kill achieved in colonies exposed to fipronil-treated donors, but the number of ants killed at 27-29°C was consistently higher. The effect of temperature on fipronil over a longer period of time cannot be determined.

We believe greater mortality was achieved at higher temperatures due to greater toxicity of these insecticides at higher temperatures to *L. humile*. Brief exposure to barriers with these active ingredients showed increased insecticidal activity (positive temperature coefficient) at higher temperatures (unpublished data). The greater mortality at higher temperatures also may be due to additive effects of temperatures stress and insecticidal action. Higher temperatures may increase ant activity, exposing more ants to the toxicant, or accelerate desiccation (Tremper 1976) or increase metabolism and render the workers more susceptible to toxic effects.

The greater level of mortality achieved by horizontal transfer with bifenthrin at 27-29°C suggests that certain active ingredients may display horizontal transfer mortality within some temperature ranges and not at others. The conditions present within ant colonies (temperature, humidity, and substrate composition) or ambient conditions may favor the effectiveness of some chemicals over others due to the added affect of horizontal transfer.

Ten ants exposed to fipronil for 1 min transferred enough fipronil to nearly eliminate a 200-worker colony in 6 d. Given the high mortality within a laboratory colony, we also might expect a great deal of horizontal transfer to occur in field populations when treated with fipronil. We suspect that the success of fipronil barrier treatments, reported by Vega and Rust (2001) may in part be due to transfer. Additional studies are warranted to demonstrate this transfer effect.  $\beta$ -Cyfluthrin and bifenthrin resulted in less mortality due to horizontal transfer with these pyrethroids may still have some limited effect on control because in the field, >10 workers are likely to be exposed.

There were no significant differences in mortality overall when adding ants dead versus alive (Fig. 1). Whereas fipronil mortality rate was significantly higher with dead donors, this did not have a significant effect on mortality over a 6-d period. Under certain circumstances, each of the various means of transfer (trophallaxis, grooming, necrophoresis, and resource contamination) may have a different level of importance depending on the nature of the nest area, resource, and environment in which treatment is delivered. For example, if ants traveled long distances to reach a treated resource, they may die before returning to the nest. In this case, horizontal transfer facilitated by necrophoresis may not likely occur. However, if they crossed a treated barrier on the way to a resource, they could potentially contaminate it. However, we did not see any evidence of this in colony boxes. With solid food resources close to the colony, the contamination due to trophallaxis may be negligible in comparison to contact with contaminated ants during necrophoresis and subsequent grooming.

Oueens were not consistently killed in these transfer studies. A dead queen was found on day 4 posttreatment in a  $\beta$ -cyfluthrin (27-29°C)-treated colony, but no other queens were found dead during the trial. At the end of the trial, two more queens were missing from the 27-29°C trial from one  $\beta$ -cyfluthrin colony and one control colony. The queens may have died and been removed or disassembled by colony workers without being observed during the trial. Queens may be less susceptible because they are larger and require a greater dose than do workers. Organization of labor within ant colonies, with older workers foraging and younger workers caring for the young and queens, would necessitate at least two transfers (old worker to young worker, young worker to queen). Distribution of different qualities of food by workers also may effect worker to queen contact (Hooper 1998). This may require very high levels of worker exposure to toxicants to reach the queen with enough active ingredient to kill her. Whatever the case, more research needs to be done to determine the mechanism by which queens were protected from horizontal transfer of the toxicants.

The persistence of the effects of horizontal transfer within a colony is not known. This may depend on the amount of ingredient that is transferred. Fipronil has an additional advantage to the pyrethroids tested by displaying a longer delay in onset of toxic effects. Insecticide barriers with longer residual activity and delayed action may result in greater numbers of ants bringing active ingredient back into the nest. Workers continued to forage across fipronil deposits with no apparent effect for 30 min (unpublished data). This allows ant workers to acquire greater dosages of fipronil and translocate it to the nest and other workers before death. The closer that barriers and treatments are to the nest, the greater the likelihood of toxicants being transferred into the nest. This study strongly suggests that more directed applications to trails and nest sites with active ingredients like fipronil would dramatically improve control.

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