Proceedings of the Fifth International Conference on Urban Pests
Chow-Yang Lee and William H. Robinson (editors), 2005.
Printed by Perniagaan Ph'ng @ P&Y Design Network, Malaysia.

HORIZONTAL TRANSFER OF BARRIER INSECTICIDES IN ARGENTINE ANTS (HYMENOPTERA: FORMICIDAE)

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Abstract Barrier applications of sprays and granules have been widely used to control Argentine ants, *Linepithema humile* (Mayr), around structures. Their activity against ants, especially the pyrethroids, has been ascribed to toxicity and repellency. Recent studies have shown that bifenthrin and cyfluthrin produced rapid knockdown of foraging ants preventing them from establishing trails across treated surfaces. Slower-acting insecticides such as fipronil provided delayed mortality and the potential for horizontal transfer. Workers exposed to simulated barriers treated with fipronil, bifenthrin, and cyfluthrin for 1 minute and returned to colony boxes provided 95% kill of the colonies in 19.8, 110.1 and 68.6 days, respectively. In a similar test with workers that had been exposed to barriers and killed by freezing, fipronil, bifenthrin, and cyfluthrin provided 95% kill of colonies in 8.2, 78.6, and 54.9 days, respectively. The mortality of unexposed ants in the colonies was as follows: fipronil > bifenthrin > cyfluthrin = control. Barriers of each insecticide were applied at label rates around structures to corroborate these findings. The barriers provided mixed results. Reduced applications and narrow barriers (0.3 by 0.3 m) of fipronil around the foundation provided about 50% reductions for 8 weeks. Thorough applications of bifenthrin and cyfluthrin provided good control for 2 and 8 weeks respectively. Field studies were consistent with the laboratory observations of insecticide transference. The studies suggest that more directed applications of fipronil and bifenthrin to trails and nest sites might provide much better control that regimented barrier applications.
Key Words *Linepithema humile*, fipronil, bifenthrin, cyfluthrin

INTRODUCTION

Barrier sprays and perimeter treatments have been widely used and recommended for ant control in and around structures in urban settings for decades (Knight and Rust, 1990; Soeprono and Rust, 2004c). Prior to the studies by Ronald Knight in the late 1980's, the label recommendations for spraying outdoors to control ants were essentially identical to those for controlling turf pests. In the past 15 years, the label directions for applying barriers have evolved and become more specific with higher treatment rates and a more precise definition of the term "barrier." Typically, thorough barrier treatments provide significant reductions in foraging ants for about 4 weeks (Rust and Knight, 1990; Rust et al., 1996; Suoja et al., 2000; Scharf et al., 2004). However, the effectiveness of barriers may be affected by various abiotic and biotic factors. Frequent irrigation and direct exposure to sunlight and to strong alkali surfaces (Wagner and Strawn, 1980; Rust et al., 1996) greatly reduced the residual activity of insecticides applied as perimeter barriers. A 7.6-cm layer of mulch required at least 140 liters of spray per 92.9 m^2 to thoroughly treat it (Bello, 1997). Biotic factors such as when the foragers contact surfaces and the substrate's temperature greatly impacted the contact activity of insecticides (Soeprono and Rust, 2004a). Lastly, the possibility of the movement of an active ingredient from one insect to another by physical contact or horizontal transfer is greatly increased in social insects such as ants.

Horizontal transfer of a toxicant may occur through member-to member contact, contact with a substrate that has been indirectly contaminated, or when oral or anal trophallaxis occurs between individual ants. Soeprono and Rust (2004b) and Wiltz et al. (2004) reported that fipronil was readily transferred from dead Argentine ants to live nestmates whereas chlorfenapyr, bifenthrin, and cyfluthrin were not as readily transferred. In both studies, the removal of dead ants to the graveyards (necrophoresis) may have contributed to the horizontal transfer. Another factor possibly contributing to horizontal transfer of insecticides is the delayed toxic action of insecticides such as fipronil. After a brief exposure to a simulated-treated barrier, the 10% survivorship percentile (SP_{10}) for fipronil ranged from 270 to 960 minutes depending upon the temperature (Soeprono and Rust, 2004a). Ants were able to maintain foraging trails across treated barriers for at least several hours increasing the number of ants contacting the barrier and allowing foragers to interact with nestmates.

The objectives of this investigation are to explore the relationships between intrinsic insecticidal activity, delayed toxicity, and horizontal transfer of insecticides between worker ants. Secondly, do these attributes predict or explain the activity of barriers under field conditions?

MATERIALS AND METHODS

Ant Colonies. Colonies were set up in plastic boxes (26 x 32 x 10 cm) with the inner sides coated with fluoropolymer resin (type 30, DuPont, Washington, WV) to prevent ants from escaping. Each box was provisioned with two polystrene weighing dishes, one containing 25% sucrose water and the other containing water. Ant condos constructed of plaster of Paris in plastic petri dishes were provided for harborage (Knight and Rust, 1990; Soeprono and Rust, 2004b). Each colony box was provisioned with 200 Argentine ant workers and two queens. The colonies were fed dead insects and allowed to acclimate for 7 days before testing.

Horizontal Transfer Studies. Ants were exposed to treated surfaces and then returned to their colony box (Soeprono and Rust, 2004b). Aqueous preparations of bifenthrin (Talstar F, FMC Corp. Philadelphia) and cyfluthrin (Tempo Ultra, Bayer Corp., Kansas City, KS) and fipronil (Termidor SC, BASF Corp., Research Triangle Park, NC) were applied to sand at recommended rates equivalent to 13.7, 10.8, and 18.8 ppm, respectively. The treated sands were allowed to dry for at least 24 hours before testing. After a 1-minute exposure, the 10 ants were returned to their original colony boxes. The number of dead ants in the colony boxes was counted daily. The dead ants were removed from the colony boxes. Each insecticide exposure was replicated three times.

In a second experiment, the ants were again exposed to treated surfaces for 1 minute. The treated ants were then placed in a freezer for 30 minutes to kill them. The 10 dead ants were then placed back into their original ant colonies. The number of dead ants was counted daily. The dead ants were removed from the colony boxes. Each of these insecticide exposures was replicated three times.

Monitoring. Efficacy provided by sprays around homes was calculated from a reduction in ant foraging, based on adjusted weight loss from monitoring tubes of sugar water before and after treatment rather than based on numbers of ants counted or trapped. Ten to twenty 15 ml polypropylene tubes (Falcon® screwcap vials) filled with about 13 ml of 50% (w/v) sucrose were placed around each structure for about 24 hours. Based on laboratory study, foraging *L. humile* imbibe an average of 0.3 mg sugar water per visit, essentially doubling their weight (Rust et al., 1996). The tubes were placed no closer that about 7 m from one another. The tubes were laid on their side, supported on a small plaster pedestal to prevent the liquid from spilling. Loss of liquid (i.e. weight) from the tubes was corrected for evaporation of liquid and drowned ants. The adjusted weight loss value made it possible to estimate the number of ant visits per station, and to map areas of greatest foraging. There is a direct correlation between amount of sugar water taken, ant visits, and the number of ants in the area. In other words, lower numbers of visits reflects lower overall ant numbers. One value of such monitoring is that it reflects long-term foraging (i.e. 24 hours) and does not depend on singular momentary observations that may vary greatly with time of day.

Only residences in which there was initially significant ant feeding at 8 or more stations were used in the study. At least 6 ml taken was considered significant. Each residence was monitored 1 week prior to treatment and 1, 2, 4 and 8 weeks after treatment.

Barrier Sprays. Applications of 0.06% bifenthrin and 0.006% cyfluthrin were made with a Honda gasoline engine-powered 194-liter FMC spray rig, at approximately 100 PSI at the pump (regulated) and through 61-m spray hose. A fan-type pattern was produced by a JD-9 spray gun equipped with a digital readout flowmeter. A target perimeter barrier application rate of 19.4 liters of diluted spray / 93.9 m^2 (5 gal / 1,000 ft^2) of treated area was maintained as closely as possible. The amount applied, as confirmed by the flowmeter, was calculated from the amount of spray needed to treat a measured perimeter swath extending approximately 61 up walls and 183 cm out. The linear distance around each home was measured before treatment in order to achieve the desired rate. Slightly less than calculated theoretical target amounts were often applied because of the presence of porches, glass doors, patios, and similar sites that precluded spraying.

Applications of 0.06% fipronil were made with a 3.8-liter B&G compression sprayer, at approximately 25 PSI at the nozzle with a coarse fan-type pattern. A target application rate of 46.5 ml of dilute spray / 93.9 m^2 of (1.5 gal. / 1,000 ft^2) of treated area was maintained as closely as possible. Because the agreed-upon barrier was only 30.5 by 30.5 cm (i.e. 1 ft on the wall by 1 ft on the ground), less than 3.8 liters of finish spray were used per residence. The volume of spray to apply was calculated beforehand from the perimeter dimensions of the homes. All treatments were applied between 0830 and 1200 hours to avoid wind drift and excessive temperatures.

Data Analysis. The mortality data of the colonies were analyzed with a probit analysis of correlated data (Thorne et al., 1995). The reductions in the number of ants at monitoring stations were analyzed with a Wilcoxon's signed-rank test.

RESULTS

The number of ants in each colony killed at 6 days was as follows: fipronil > bifenthrin > cyfluthrin = control. Ants exposed to fipronil and returned to the colony provided faster kill of colony nests than did either bifenthrin or cyfluthrin (Tables 2 and 3). The dead ants with fipronil (slope = 0.348 ± 0.0226) returned to the colonies provided significantly faster kill of the colonies than did live ants exposed to fipronil (slope = 0.123 ± 0.0131; t = 8.619, P<0.05).

Thorough applications of fast-acting barriers such as bifenthrin and cyfluthrin provided significant reductions for 8 and 2 weeks, respectively (Table 1). The reduced application rates and narrow barrier at the base of the structure of fipronil provided consistent reductions in the number of ant visiting monitoring stations for 8 weeks.

Table 1. Performance of perimeter sprays against Argentine ant, *Linepithema humile.*

Insecticide	Avg. Spray Vol. Applied (ml)	Ant Visits Avg. Precount	Avg. No. Ant Visits/vial (% reduction)[a]			
			7	14	30	60
Fipronil, 0.06%	2,293	34,011	14,402 (66.5)*	10,610 (68.8)*	12,647 (62	4,418 (57.6)*
Bifenthrin, 0.03%	38,986	31,895	6,452 (79.8)*	5,942 (81.3)*	5,267 (83	9,432 (70.4)*
Cyfluthrin 0.006%	24,981	27,217	9,094 (66.6)	7,938 (70.8)	16,347 (39.[illegible]	14,981 (45.0)

[a] 10 – 20 monitoring vials were placed around the structures. * indicates significant reductions at P <0.05 (Wilcoxon signed ranks tests).

DISCUSSION

Barrier treatments have been widely used against Argentine ants in urban and agricultural settings (Soeprono and Rust, 2004). Their activity had been attributed to contact insecticidal activity and repellency (Knight and Rust 1990). Addison (2002) concluded that the most likely explanation for the effectiveness of permethrin, cyfluthrin, and chlorpyrifos barriers applied to the stems of grape vines was that they resulted in ant mortality. Similarly, Pranschke (2003) found that barriers of bifenthrin granules were non-repellent to red imported fire ants, *Solenopsis invicta* (Buren), suggesting that it killing forager scouts preventing trails from establishing. High mortality was observed of foragers crossing the barrier. Recent studies by Soeprono and Rust (2004a) have shown that fast-acting pyrethroid barriers prevent recruitment and thus give the appearance of being repellent. Thus, the speed of insecticidal activity is probably an extremely important criterion of barriers.

When foraging ants contact chemical barriers, sufficient insecticide must be available on contact if the ant receives a lethal dose. Numerous factors such as physical and chemical nature of the substrate impact the amount of insecticide available for uptake by crawling insects. Wagner and Strawn (1980) showed that the residual activity of most of the carbamates and organophosphate insecticides tested against Argentine ants was severely reduced after 1 month especially on substrates like concrete. Organic materials such as mulch and foliage interfere with the application and residual activity of insecticides (Bello, 1997). Delayed toxicity or the reduced speed of insecticidal activity is extremely important to permit behaviors such as grooming and trophallaxis to occur after exposure to an insecticidal barrier. Consequently, the availability of an insecticide, its inherent toxicity, and its speed of activity are the primary factors determining if the horizontal transfer of insecticides from barriers to other ants is feasible.

Table 2 shows the potential contact activity of several insecticides against cat fleas (Moyses and Gfeller, 2001) and an estimated amount required to kill Argentine ants. If penetration across the cuticle is similar in both species, then < 1 ng of fipronil, deltamethrin or imidacloprid will kill 50% of the ants treated. Ants contacting treated barriers must acquire at least this amount of insecticide to be killed. More importantly, it is the minimal amount that must be transferred to other nestmates to kill them. Consequently, the donor ants must be exposed to considerably higher dosages if transference is to occur.

Table 2. Toxicity of various insecticides topically applied to adult cat fleas, *Ctenocephalides felis* (Bouchè), corrected for the weight of Argentine ants (adapted from Moyses and Gfeller 2001).

Insecticide	**LD_{50} cat flea (ng/insect)**	**LD_{50} Argentine ant[a] (ng/insect)**
Fipronil	0.18	0.39
Deltamethrin	0.19	0.41
Imidacloprid	0.39	0.84
Cypermethrin	1.4	3.01
Diazinon	6.2	13.33
Permethrin	7.2	15.48

[a] Cat fleas avg. weight (± SD) = 0.20 ± 0.043 mg (n = 75). Argentine ant workers avg. weight (± SD) = 0.43 ± 0.024 mg (n = 75). LD_{50} Argentine ant = LD_{50} cat flea x 2.15.

Table 3. Efficacy of fipronil, 0.01% and imidacloprid, 2.15% gel baits against American cockroaches in sewers in Riverside, California (USA).

		% Reduction at month[a]			
Treatment[b]	Avg. before	3	6	9	12
Fipronil, 0.01% gel	67	97.0	13.4	25.4	17.9
Imidacloprid, 2.15% gel	96	98.6	100	91.7	90.6
Untreated	153	0.0	0.0	0.0	0.0

[a] Average number cockroaches in 8 manholes before and after. Reductions based on visual counts.
[b] Manholes first baited September 2003 and re-baited if no bait remained at time of counting.

Bifenthrin and cyfluthrin provided rapid knockdown and kill of ants even after brief exposures of 1 minute (Soeprono and Rust, 2004a). Within 10 minutes after exposure, 90% of the ants were killed. Even deposits with as little as 2-3 ppm provided 90% kill within 35 minutes. Fipronil provided significantly slower kill than did the pyrethroids. Similar deposits required 270 and 960 minutes, respectively, to kill 90% of the ants. The delay in toxicity permits ants to continue recruiting additional workers, return to the nest, and interact with other workers after being exposed to fipronil barriers.

In our study, nestmates contacting live or dead ants exposed to fipronil were killed within 20 days. Bifenthrin produced considerably lower mortality. The faster rate of kill of nestmates in the colonies of ants exposed to dead ants with fipronil, suggest that necrophoresis (taking dead to graveyards) is extremely important in transferring the insecticide. The delayed toxicity of fipronil may contribute to necrophoresis if exposed workers die within or nearby the colony. Unexposed workers in the colony contact dead foragers in their daily maintenance routines, increasing their likelihood of being exposed to lethal doses of fipronil. Ants exposed to fast-acting insecticides die away from the colony and are less likely to be involved in necrophoresis.

Extremely thorough barrier sprays with pyrethroids such as bifenthrin, lambda-cyhalothrin, and deltamethrin provided > 80% reductions in ant counts for up to 8 weeks (Klotz et al., 2002). Rust et al. (1996) found that only combination sprays and granular applications of cyfluthrin provide > 80% reductions for 8 weeks. Chlorpyrifos, cypermethrin and permethrin sprays failed to provide > 30 days controls. There were occasions with pyrethroids where ants were trapped inside the barrier (Rust et al., 1996, Gulmahamad, 1997). Barriers of the so-called "non-repellent" fipronil provided 8 weeks effective control of ants around structures (Scharf et al., 2004). However, an increase in the numbers of odorous house ants, *Tapinoma sessile* (Say), in treated barriers suggested that fipronil barriers had an immediate impact on ant activity, but that the residual effectiveness of the barriers may have been decreasing.

In our field studies, the barriers bifenthrin and cyfluthrin performed as expected. Similar barrier applications of fipronil have provided outstanding control (99% reductions) for up to 4 weeks (Vega and Rust, 2003). The reduced application rates of fipronil provided consistently significant reductions for up to 8 weeks. In part, horizontal transfer must be occurring considering the reduced amount of fipronil applied in a narrow band around the structure. Additional research using dyed ants similar to the Vega and Rust study (2003) is needed to confirm the potential transfer and its impact on control.

To exploit the potential horizontal transfer of barrier insecticides, more directed and precision applications of these insecticides are needed. The ant recruitment trails, satellite nests, and main nests need to be targeted. Additional testing is warranted to support the necessary changes in the label recommendations and definitions of perimeter treatments to control Argentine ants. In this manner, the amount of insecticide applied around structures can be greatly reduced.

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