HOUSEHOLD AND STRUCTURAL INSECTS

Method of Insecticide Delivery Affects Horizontal Transfer of Fipronil in the German Cockroach (Dictyoptera: Blattellidae)

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ABSTRACT Horizontal transmission of insecticide occurs when foragers contact or ingest an insecticide, return to the aggregation or nest, and translocate the insecticide to the shelter and its vicinity. Relatively more sedentary members of the population then contact or eat the translocated insecticide and die. We evaluated three different methods of delivering fipronil to adult male German cockroaches, Blattella germanica (L.), for their potential to cause such secondary mortality in various developmental stages of the cockroach. Adult males topically treated with 5 ng of fipronil (\approx LD₉₉) caused low mortality in untreated nymphs and no mortality in untreated adults within the same aggregation. Males exposed to residual fipronil on a glass surface translocated more insecticide, resulting in higher mortality of cockroaches they contacted, but only early instars were affected and no adult mortality was observed. Ingested fipronil bait, however, was most effectively translocated, and caused high mortality of untreated adults and nymphs. Ingestion of fipronil also caused greater secondary kill compared with a topical application of 25 ng, approximately the same amount recovered from the exterior of males that ingested 1 mg of 0.05% fipronil bait. Secondary mortality in the untreated population was significantly affected by the duration of contact between the treated and untreated cockroaches, the quantity and freshness of excretions from the treated insects, and the accessibility of the secretions to untreated cockroaches. The mechanisms that cause secondary kill may include ingestion of excreted fipronil residues, cannibalism of bait-fed cockroaches, as well as contact with fipronil-contaminated substrates.

KEY WORDS Blattella germanica, fipronil, translocation, horizontal toxicant transfer

THE SEARCH FOR new insecticides and new methods of insecticide delivery to control the German cockroach, Blattella germanica (L.), continues because this insect remains one of the most economically and medically important pests of the urban environment (Brenner 1995). In this ongoing process, older chemicals are displaced because of insecticide resistance, increasingly strict regulations, and public demand for safer and more effective products. New active ingredients and innovative delivery tools emerge to provide effective means of dealing with infestations. One of the newest insecticides is fipronil, a fast-acting phenylpyrazole that blocks the transmission of signals by the inhibitory neurotransmitter γ -aminobutyric acid (Colliot et al. 1992, Cole et al. 1993, Moffat 1993). It is highly toxic to the German cockroach (Kaakeh et al. 1997a) and other pests of public and medical importance (Scott and Wen 1997).

Baits have become popular and effective formulations against urban insect pests. Compared with residual sprays, baits offer the advantages of long residual activity, safer application, and reduced environmental pollution (Cornwell 1976, Reierson 1995). Social or gregrarious insects, such as termites, ants, and cockroaches are particularly susceptible to baiting because the foragers return to the nest or aggregation and contaminate it and its vicinity with traces of the insecticide. Relatively fewer mobile members of the

population then receive the insecticide through contact, trophallaxis, coprophagy, or necrophagy, as demonstrated for populations of the German cockroach in laboratory and field settings (Silverman et al. 1991; Kopanic and Schal 1997, 1999; Kaakeh et al. 1997b; Gahlhoff et al. 1999). Coprophagy was the major mechanism mediating horizontal transfer of hydramethylnon, especially in first instars (Silverman et al. 1991; Kopanic and Schal 1997, 1999). However, fipronil exhibits extremely high contact toxicity to insects and it intoxicates the German cockroach much faster than hydramethylnon (Kaakeh et al. 1997a). Therefore, even if ingested fipronil is not excreted by defecation before death, mechanisms other than coprophagy, such as contact with dead cockroaches, could be involved in the transfer of fipronil among cockroaches.

We examined the horizontal transmission of fipronil among cockroaches by using three different insecticide delivery methods: topical, residual, and oral (ingestion). First, we determined the toxicity of fipronil to adult male cockroaches by using these methods of delivery. We then used a sequential design in which treated adult males could transfer the insecticide to untreated adults and nymphs. Last, we investigated the mechanisms by which the fipronil residues were dispersed within a cockroach population.

Materials and Methods

Insects. Insecticide-susceptible cockroaches that originated from American Cyanamid (Princeton, NJ) were reared at 27°C, ambient relative humidity, a photoperiod of 12:12 (L:D) h, and provided with water and Purina Rat Chow #5012 (Purina Mills, St. Louis, MO). Cockroaches were used early at each developmental stage during the peak feeding period of adult females (Cochran 1983, Hamilton and Schal 1988) and nymphs (Valles et al. 1996, Young and Schal 1997). Adult females and first and second instars were collected from synchronous cultures within 12 h of ecdysis. Adult males, 10–14 d old, were used to vector the insecticide to other cockroaches.

Topical Application. Toxicity of fipronil was determined by topical application with a microapplicator equipped with a 50-μl syringe (Hamilton, Reno, NV). Technical grade fipronil (97.1% [AI]; Rhône-Poulenc, Research Triangle Park, NC) was delivered in 0.5 µl of pesticide grade acetone (Fisher, Pittsburgh, PA) to the ventral mesothorax of CO2-anesthetized adult male cockroaches. Cockroaches were treated with five doses of fipronil (1, 2, 3, 4, and 5 ng per insect) and control males were treated with acetone. Each dose was replicated four times, with 10 insects per replicate. Treated males were placed in 150 by 25-mm plastic petri dishes, provided with food and water, and monitored for mortality for 72 h at 27°C. If insects on their backs were unable to right themselves when prodded they were considered dead.

Transfer of fipronil among cockroaches was evaluated by topically treating each of 10 adult males with either 5 ng of fipronil (\approx LD₉₉) or 25 ng of fipronil. Upon recovery from the anesthesia the males were released into a population consisting of 10 adult males, 10 adult females, 10 first instars, and 10 second instars housed in either small cages (19 by 14 cm; replicated six times) or large cages (53 by 29 cm; replicated five times). Insects were provided with food, water, and a cardboard shelter, and maintained under the same ambient conditions as the colony. The treated and untreated cockroaches were then allowed to interact freely for 6 d and mortality was recorded twice daily.

Contact Toxicity. Fipronil was applied in acetone to the bottom of glass crystallizing dishes (9.5 cm in diameter) and the solvent was evaporated while the dishes were gently rotated to facilitate a uniform distribution of fipronil. The sides of the dishes were coated with a thin layer of petroleum jelly, which restricted the insects to the treated surface. Three doses of fipronil were evaluated (2.5, 1.25, and 0.625 $\mu g/cm^2$), adult males were allowed to walk out of test tubes onto the treated surfaces, and they were exposed to these residues for 15, 30, 60, 120, or 240 s. One replicate of 20 males was performed for each dosetime of exposure combination. After exposure, the insects were transferred without anesthesia to clean 150 by 25-mm petri dishes, provided with food, water, and a shelter, and returned to the incubator. Mortality was recorded daily for 4 d after exposure to fipronil. To evaluate transfer of fipronil among cockroaches, males whose wings were partially clipped for recognition were placed for either two or 10 min on a glass surface treated with one of the three doses of fipronil. They were then released among untreated cockroaches in a small cage (19 by 14 cm). The insects were provided with food, water, and a cardboard shelter. Mortality of untreated cockroaches was recorded daily for 4 d.

Oral Toxicity. Baits containing various concentrations of fipronil were formulated by mixing finely ground rat chow (5 g) with an acetone solution of fipronil (2.5 ml) to form 0.05, 0.005, 0.0005, and 0.0001% baits (% fipronil, wt:wt). The acetone was evaporated in a fume hood for 24 h with intermittent mixing; when the solvent had completely evaporated, the mixture was vigorously vortexed. Twenty adult males were placed in a 150 by 25-mm plastic petri dish, provided with water and a shelter, and starved for one scotophase (12 h). At the onset of the following photophase the insects were provided the bait continuously without other food. Mortality was recorded at 1-to 6-h intervals for 78 h.

In experiments on horizontal transmission, 10 adult males were starved for one scotophase, and then fed 0.05% (fipronil) Maxforce FC bait (Clorox, Oakland, CA) for 2 h. They were then transferred to clean 19 by 14-cm plastic cages together with an untreated population of 10 adult males, 10 adult females, 10 first instars, and 10 second instars, and provided with water, rat chow, and a shelter. We also designed various bioassays to investigate the relative importance of the initial contact between the treated and untreated cockroaches and the role of various excretions in the transfer of fipronil. It is important to note that treated males first exhibited symptoms of poisoning $\approx 3-4$ h after they ingested fipronil baits. Six replications of each experiment were performed in 19 by 14-cm cages.

Control (Continuous Exposure). This experiment was designed to establish a maximum level of secondary mortality in a mixed population of *B. germanica* exposed to 10 males that ingested fipronil bait. Males were fed the bait for 2 h and then immediately transferred into the experimental cage with untreated cockroaches.

Role of Fresh Excretions. After feeding on the bait for 2 h, the males were placed in a cage provisioned with rat chow, water, and a cardboard shelter. After 6 or 24 h, when all the treated males were moribund or dead, untreated cockroaches were introduced into the cage and allowed to freely contact the treated males and residues they deposited.

Role of Deposited Residues. This experiment was designed to exclude interactions with the treated males and investigate the role of the excreted fipronil residues alone. After feeding on the bait for 2 h, the males were placed in cages provisioned with rat chow, water, and a cardboard shelter. After 6 or 24 h the treated males were discarded and untreated cockroaches were introduced into the cage and allowed to freely contact residues deposited by the treated males.

In all bioassays, rat chow, water, and a shelter remained in the cage throughout the experiment.

Statistical Analysis. LD₅₀ determinations of topically applied fipronil were made by using probit analysis (SAS Institute 1997). Preliminary analysis (CAT-MOD, SAS Institute 1997) revealed that mortality of males, females, first and second instars was different after exposure to males treated with three different concentrations of residual fipronil. Contingency table analysis (PROC FREQ, SAS Institute 1997) was then performed to determine whether there were significant differences in the survivorship of cockroaches exposed to adult males that were treated with different concentrations of fipronil. This analysis compared frequencies of dead and live cockroaches for each of the concentration/time of exposure. When significant chi-square values (P < 0.05) were obtained, a posthoc Z-test (Marascuilo and McSweeney 1977) on proportions of dead cockroaches was performed to identify the significant treatment differences. Nonparametric analyses were performed using the Mann-Whitney U test (StatView 1998) to analyze differences between two samples. Data are presented as means \pm SE. All statistical analyses were conducted at the $\alpha = 0.05$ level of significance.

Results and Discussion

Topical Application and Horizontal Transmission. Fipronil killed adult male German cockroaches in nanogram quantities per insect. At 72 h after topical application, the slope of the probit transformed mortality data was 2.91 \pm 0.54, the LD₅₀ was 2.40 ng/male (95% FL, 1.73, 2.98) and the LD₉₅ was 4.23 ng/male (95% FL, 3.93, 15.01). No mortality was recorded in any of the control insects that received acetone alone. The fresh body mass of 14-d-old males was 56.1 \pm 0.5 mg (n=50).

Ten adult males were treated with 5.0 ng of fipronil per insect and immediately placed into a large cage $(1,537~\rm cm^2)$ containing a mixed population of nymphs and adults. The treated males and untreated cockroaches occupied the same shelter and consumed the same food and water. Despite the proximity of treated and nontreated insects, no mortality was observed in either nymphs or adults of the latter after 5 d (n=5 replicates with 20 adults and 20 nymphs each). In smaller cages $(266~\rm cm^2)$, some of the first instars $(33.3\pm3.3\%,\ n=6$ replicates) and second instars $(21.7\pm0.5\%,\ n=6$ replicates) died when exposed to adult males treated with 5 ng of fipronil. However, no adults died in the small cages in any of the replicates.

To test whether higher doses of topically applied fipronil would kill more of the untreated cockroaches we treated adult males with 25 ng of fipronil. A dose of 25 ng was chosen because previous experiments with radiolabeled fipronil showed that after 10 min on a glass surface treated with 2.5 μ g/cm² an adult male retained 27.0 \pm 2.58 ng fipronil (n=10 replicates) (G.B., unpublished data). We also determined that after ingesting 1 mg of 0.05% radiolabeled fipronil bait an adult male excreted 22.2 \pm 4.70 ng of fipronil (n=1)

10 replicates) or 4.4% of the ingested radiolabel. This extrapolation assumes that all the excreted radiolabeled material was fipronil or metabolites of equal toxicity, a reasonable supposition in light of results showing that inhibitors of fipronil metabolism may diminish toxicity of fipronil (Scott and Wen 1997, Valles et al. 1997). Therefore, the 25-ng topical dose is representative of the amount of fipronil that might typically be found on a cockroach that has died from exposure to surface-applied fipronil (2.5 μ g/cm²) or from ingesting 0.05% fipronil bait. However, the higher dose of topically applied fipronil did not facilitate greater horizontal transfer in the small cages. Mortality was still low, $36.7 \pm 0.2\%$ for first instars, $20.0 \pm 0.3\%$ for second instars, and none in adults. The secondary mortality caused by 5 and 25 ng was not significantly different in either the first instars (Mann-Whitney *U* test; Z = 0.641, n = 6, P = 0.52) or second instars (Z = 0.400, n = 6, P = 0.69).

First- and second-instar *B. germanica* have been shown to forage shorter distances than older nymphs and adults (Sommer 1975, Cloarec and Rivault 1991, DeMark et al. 1993). It is possible that in the large cages nymphs failed to interact extensively with fipronil-treated adults, especially because many of the treated males died outside the shelter and away from the food and water, and their excretions would be less available to small nymphs. In small cages, more of the treated males died within or in the vicinity of the shelter, thus increasing the probability that fipronil would be transferred to early instars through contact with traces of the insecticide. Overall, however, topically applied fipronil caused only low secondary mortality of nontreated insects.

Contact Toxicity and Horizontal Transmission. Several factors, such as the application rate, formulation, surface, and most importantly, the length of time a cockroach contacts the treatment, affect the performance of residual insecticides (Cornwell 1976, Chadwick 1985, Wickham 1995). Male mortality was affected by both the dose and duration of exposure to fipronil on a glass surface. A 2-min exposure to 1.25 or $2.5 \mu g/cm^2$ killed all males within 24 h (Fig. 1D). Shorter exposures of 1 min and 30 s to the same residues, however, required 48 and 72 h, respectively, to kill all cockroaches (Fig. 1 B and C). Very brief exposures of as little as 15 s to 1.25 and 2.5 $\mu g/cm^2$ fipronil killed all cockroaches in 96 h (Fig. 1A). Overall, all three doses of fipronil were effective in killing adult males and even the lowest concentration (0.625) μg/cm²) resulted in 100% mortality when cockroaches were exposed to it for 4 min.

Cockroaches exposed to a fipronil-treated glass surface translocated the insecticide more efficiently to untreated nymphs than topically treated males (Mann–Whitney U test, Z=4.157, n=6, P<0.001). Nevertheless, no untreated adults died even when housed with males that were exposed to $2.5~\mu g/cm^2$ for 10~min, a treatment that killed 100% of first instars and 95% of second instars (Table 1). With less exposure (2 min) of adult males to a lower concentration of fipronil $(0.625~\mu g/cm^2)$, significantly fewer first instars

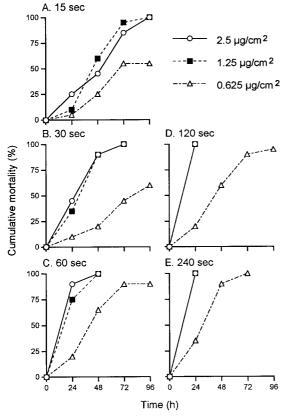


Fig. 1. Cumulative daily mortality in adult males exposed to fipronil residues on a glass surface. Twenty males were exposed for each time-dose combination and monitored for 96 h in clean 150 by 25-mm petri dishes provisioned with food and water.

died (Student's t-test, t = 25.00, n = 6, P < 0.001) and all second instars and adults survived. Blatta orientalis L., too, picks up a high dose of cypermethrin during short forays, exhibits symptoms within 1 h, returns to the shelter and transfers the insecticide to other members of the population (le Patourel 1998). Horizontal transfer of cypermethrin also declines in larger arenas as fewer cockroaches return to the shelter.

Table 1. Cumulative percentage mortality of untreated B. germanica nymphs exposed for $4\,\mathrm{h}$ to adult males that were treated with residual fipronil

Fipronil concentration	Duration of male exposure (min)	No. of replicates	% mortality		
on glass $(\mu g/cm^2)$			First instars	Second instars	
2.5	10	5	$100.0 \pm 0.0a$	$95.0 \pm 2.2a$	
2.5	2	8	$85.0 \pm 1.9b$	$58.8 \pm 4.4b$	
1.0	10	5	$98.0 \pm 2.0ab$	$84.0 \pm 5.1a$	
1.0	2	6	$45.0 \pm 8.9 \mathrm{c}$	$5.0 \pm 3.4c$	
0.625	2	6	$16.7 \pm 3.3d$	$0.0 \pm 0.0c$	

Means \pm SE followed by different letters within each column are significantly different based on a Z test ($\alpha=0.05$). In each assay an untreated population of 10 males, 10 females, and 20 nymphs was used with 10 treated males in a 19 by 14-cm cage.

Although topical applications delivered fipronil to the ventral mesothorax between the coxae, it would seem that the ventral thorax would be sufficiently exposed after the treated males died ventral side up. Still, secondary mortality was minimal after topical application. In contrast, males that walked on a fipronil-treated surface probably picked up insecticide on their tarsi and transferred it through grooming to other parts of the body. Nymphs that contacted treated cockroaches were probably more likely to acquire the insecticide because of its greater bioavailability.

Bait Ingestion and Horizontal Transmission. All baits containing >0.0001% fipronil in rat chow killed 100% of the adult males in <24 h. With continuous access to 0.05% fipronil all males died in 4 h, the 0.005% bait took 7 h, and the 0.0005% bait took 17 h. On average, 14% of males that ingested the 0.0001% fipronil bait died after 78 h and none of the males that were fed rat chow died.

After ingesting the Maxforce FC bait (0.05% fipronil) adult males effectively translocated the insecticide and caused the highest secondary mortality in both nymphs and adults. In control assays that provided for continuous interaction among the bait-fed males and the untreated insects all first and second instars died within 7 d (Table 2). In contrast to the topical and residual treatments, which failed to kill untreated adults, 85% of the adult males and 48.3% of the adult females died from exposure to fipronil-fed males and their excretions. The mechanisms underlying secondary kill may include cannibalism, coprophagy, or transfer of oral or anal excretions through contact or ingestion.

Cannibalism occurs in the German cockroach (Roth and Willis 1960, Guthrie and Tindall 1968) and secondary kill can occur in laboratory assays when starved adult males cannibalize smaller bait-fed nymphs, including nymphs fed fipronil baits (Gahlhoff et al. 1999). Nevertheless, the untreated population of cockroaches was not starved and we saw no evidence of cannibalism in our assays. Likewise, it would appear that coprophagy played a minor role in fipronil baitinduced secondary kill. In the ≈4 h before they died, males excreted little, and in most cases, no feces in the cage. In addition, experiments with radiolabeled fipronil showed that no ingested fipronil was recovered in the feces and most of the excreted fipronil was eliminated as liquid excretions from the mouth (G.B., unpublished data). Conversely, cockroaches that ingested the slow-acting hydramethylnon defecated extensively before they died, their feces contained hydramethylnon, and coprophagy resulted in extensive secondary kill (Silverman et al. 1991; Kopanic and Schal 1997, 1999). Indeed, coprophagy appears to be adaptive in B. germanica, providing small nymphs with nutrients and possibly microbial symbionts (Kopanic et al. 2001).

We suggest that ingested fipronil is made uniquely available to untreated cockroaches through liquid excretions from bait-fed cockroaches. The bait-fed males regurgitated some of the bait (visible as clear droplets), excreted anal fluids that contained fipronil, and

Table 2. Cumulative 7-d percentage mortality of untreated B. germanica adults and nymphs exposed to bait-fed adult males

Experimental design		Secondary mortality (% \pm SE)				
Contact with bait-fed males ^a	Contact with fipronil- containing excretions	Adult males	Adult females	First instars	Second instars	
Continuous	Continuous	85.0 ± 5.0a	48.3 ± 6.0a	$100.0 \pm 0a$	100.0 ± 0a	
After 6 h	After 6 h	$38.3 \pm 1.0b$	$15.5 \pm 3.4b$	$100.0 \pm 0a$	$96.7 \pm 2.1a$	
None	After 6 h	$0.0 \pm 0c$	$0.0 \pm 0c$	$45.0 \pm 2.2b$	$31.7 \pm 4.0c$	
After 24 h	After 24 h	$18.3 \pm 3.1b$	$0.0 \pm 0c$	$88.3 \pm 7.9a$	$60.0 \pm 5.8b$	
None	After 24 h	$1.7 \pm 1.7c$	$0.0 \pm 0c$	$41.7 \pm 3.1b$	$33.3 \pm 3.3c$	

Means followed by different letters within each column are significantly different based on a Z test ($\alpha = 0.05$). For each of six replicates 20 untreated adults and 20 untreated nymphs were used with 10 treated males.

 \approx 4.5% of the radiolabeled fipronil that was ingested was subsequently collected from the exterior of the dying cockroach (G.B., unpublished data). Because the LD₅₀ for adult males is only 2.4 ng per insect, the 22.2 ng of fipronil (=4.5%) excreted from ingesting 1 mg of 0.05% fipronil bait represents a relatively large dosage for adults and certainly for small nymphs. Still, because the mass of ingested fipronil was no different from that after topical application or exposure to a treated surface, we hypothesized that fipronil, or the matrix in which it was excreted, was qualitatively different from that available after topical or contact treatment. As suggested earlier, the excreted metabolites may indeed be more toxic than fipronil due to enzymatic activation within the digestive tract. In addition, we suggest that excreted fipronil may promote secondary mortality because it is mixed in an attractive or palatable regurgitate and therefore it may be in a more bioavailable liquid form. We observed nymphs and adults feeding on excretions from dead and dying males and time-lapse video records showed that first instars were highly attracted to moribund cockroaches even when provisioned with rat chow and water (G.B., unpublished data). These observations prompt us to speculate that the fipronil-induced excretion was both attractive and palatable to nymphs and it might serve to release fipronil more slowly as the cockroach dies. Le Patourel (1999) reported that adult females and third-instar B. orientalis died when they were exposed in jars to the residues of fipronil-fed females. Mortality in the secondary population was related to the amount of bait ingested by the females, presumably because females that ingested more bait regurgitated more fipronil.

To evaluate the role of exudates that emanate from dying cockroaches and residues deposited after bait ingestion we exposed cockroaches to fresh and older residues from fipronil bait-killed cockroaches, with and without contact with the moribund or dead cockroaches. We found that in contrast to the high secondary mortality in cockroaches that were continuously exposed to dying males and their residues, no adults and <50% of the nymphs died when the dead males were removed from the cage (Table 2). These results confirm that little fipronil is translocated in residues that are not directly associated with the dying cockroach. Moreover, mortality of untreated cock-

roaches was significantly lower when they were initially prevented from contacting the fipronil-fed males and the residues they excreted (Table 2). By delaying contact between treated and untreated cockroaches for 6 h, secondary mortality of nymphs was unaffected, but adult mortality declined by >55%, suggesting that fresh residues, primarily on the dying cockroach, caused greater secondary mortality. This trend was even more apparent when the untreated cockroaches contacted dead males and their residues 24 h later. Secondary mortality was significantly diminished in all cockroaches except in first instars (Table 2). This suggests that the effectiveness of excreted fipronil residues quickly diminishes over time as these deposits dry and adhere to the dead cockroaches and the substrate. Although these deposits may retain some insecticidal activity due to fipronil's high contact activity, they may not be eaten by other cockroaches and consequently do not kill adults.

These results support the hypothesis that ingested fipronil is primarily associated with the dying cockroaches and not with fecal or other deposits. This is in sharp contrast to ingested hydramethylnon, residues of which are found in copious amounts in the feces (Silverman et al. 1991; Kopanic and Schal 1997, 1999), facilitating secondary mortality through coprophagy. The superiority of fresh excretions, coupled with their intimate association with moribund cockroaches, strongly implicate the dying cockroach as a delivery vehicle of attractive, fipronil-containing excretions to cockroach aggregations. Although ingested fipronil incapacitates cockroaches within just 3-4 h, and most of their fipronil-containing excretions accumulate in the first 6 h, the dying cockroaches continue to excrete fipronil, albeit at lower rates, for >12 h (G.B., unpublished data).

What remains to be shown in this and other species is whether fipronil is chemically altered in excretions, the source and time course of the excretion(s), its relative attractiveness and palatability, and what mechanisms are involved in facilitating secondary kill. The importance of fresh excretions and loss of efficacy of older (dry) excretions both suggest that ingestion, more than contact, mediate secondary kill. Our video analysis is consistent with this supposition and further indicates that moribund cockroaches are highly attractive to first instars. Because the translocated fipro-

[&]quot;Contact with bait-fed adult males or their excretions was manipulated by introducing the untreated adults and nymphs 0, 6, or 24 h after the bait-fed males were placed in the 19 by 14-cm cage.

nil is associated with dying cockroaches, and young nymphs are relatively sedentary, the time-course of ingesting fipronil bait, returning to the shelter, and subsequent interactions with other cockroaches will profoundly affect the extent of secondary kill.

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