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Emetophagy: Fipronil-Induced Regurgitation of Bait and Its Dissemination from German Cockroach Adults to Nymphs

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Blattella germanica (L.) (Dictyoptera: Blattellidae) that were fed fipronil bait produced liquid excretions that were toxic to conspecifics. We have used a combination of analytical and behavioral assays to localize the source, to elucidate the time course, and to evaluate the role of these excretions in facilitation of secondary kill. Fipronil excretion coincided with the onset of the paralytic symptoms, and most of the excreted fipronil (79%) was eliminated during the first 12 h after ingestion of the bait. More than 74% of the total radioactivity excreted in 48 h from [^{14}C]fipronil-fed females was recovered from their oral region, and time-lapse video analysis showed that first instars were highly attracted to these excretions. Moreover, first instars preferentially contacted the oral region of dying females and imbibed the liquid exudates. Emetophagy, the ingestion of insecticide-induced regurgitate, may constitute an important mechanism by which fast-acting, emetogenic insecticides are disseminated within cockroach populations.

Key Words: Blattella germanica; emetophagy; fipronil; horizontal toxicant transfer; bait; trophallaxis.

INTRODUCTION

The German cockroach, *Blattella germanica* (L.), exhibits several behavioral adaptations that facilitate its successful exploitation of human-built structures (1). Its foraging behavior, for example, is well adapted to both its gregariousness and the patchy distribution of its resources. Like other commensal species of cockroaches, it is an omnivorous scavenger and engages in facultative coprophagy (ingestion of feces), necrophagy (ingestion of dead conspecifics), and cannibalism. The German cockroach lives in aggregations where relatively sedentary neonates live in close proximity to older, more mobile nymphs and adults. When food deprived, these facultative behaviors have significant nutritional benefits for survival of the German cockroach, they might allow it to reclaim vital nutrients with minimal foraging (2), and they promote faster development and reproduction (3). These behaviors (aggregation, coprophagy, necrophagy, cannibalism), however, can also have severely detrimental effects because they can disseminate pathogens and insecticides within cockroach aggregations. Foraging members of a cockroach population can translocate insecticides, particularly as baits, to other members of the aggregation, which in turn ingest insecticide-laden feces, other excretions, or dead cockroaches. Indeed, horizontal transmission of baits has been demonstrated to occur through coprophagy (4–8) and necrophagy (9–11) in laboratory and field settings.

In studies on the horizontal transmission of fipronil, a fast-acting neurotoxic insecticide, we have shown that fipronil excreted from bait-fed *B. germanica* caused secondary mortality in untreated cockroaches within the same cage (12, 13). Presence of dying foragers and contact between them and untreated cockroaches facilitated the distribution of fipronil and caused high levels of mortality in untreated cockroaches. Their feces, on the other hand, appeared to play a minor role in the transfer of fipronil, in contrast to that of hydramethylnon, a delayed-action insecticide that is transferred by coprophagy (4, 7, 8). Cockroaches that ingested fipronil exuded

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liquid excretions that appeared attractive and lethal to conspecifics within the same aggregation, suggesting that multiple mechanisms may facilitate the sharing of fipronil bait among cockroaches.

In this study we report results of bioassays with radiolabeled fipronil to identify the sources of excreted fipronil. Time-lapse video observations examined the responses of first instars to fipronil-killed cockroaches. From both analytical and behavioral approaches we conclude that regurgitates, and to a lesser extent anal excretions, play an important role in horizontal dissemination of insecticides in *B. germanica* aggregations.

MATERIALS AND METHODS

Insects

Insecticide-susceptible cockroaches that originated from American Cyanamid (Princeton, NJ) were reared at 27°C, with variable ambient relative humidity and a photoperiod of L12:D12 h, and provided with water and Purina Rat Chow (No. 5012, Purina Mills, St. Louis, MO). Cockroach nymphs were used within 24 h of ecdysis, during their peak feeding period (14, 15). Two-day-old adult females were used because these vitellogenic females feed maximally (16, 17).

Fipronil Ingestion

[^{14}C]Fipronil (25.6 mCi/mmol) was a gift from Rhône-Poulenc (now Aventis, Research Triangle Park, NC). It was dissolved in spectrophotometric-grade acetone of which 0.2 μl (60,000 dpm = 465 ng fipronil) was applied to a 1-mg piece of fresh bread with a microapplicator equipped with a 10-μl syringe (Hamilton Co., Reno, NV). In experiments with nonradiolabeled fipronil, Maxforce FC (0.03% fipronil) bait stations (Clorox Co., Oakland, CA) were used.

Time Course of Fipronil Excretion in Bait-Fed Cockroaches

Excretion of fluids by fipronil-fed cockroaches was commenced at approximately the time when paralytic symptoms first appeared and continued for some time thereafter. To determine a precise time course of fipronil excretion, 2-day-old adult females were starved for 24 h and then fed the radioactive bait (60,000 dpm fipronil), and only females that consumed the entire 1 mg bait were used subsequently. Each female was transferred to a 7.5-ml glass vial without food or water. Twelve or 24 h later, dead cockroaches (n = 20) were removed from the vials and the amount of fipronil excreted within the vials was determined in a Beckman LS 5801 liquid scintillation spectrometer (Beckman Instruments, Irvine, CA). The amount of fipronil excreted 12 to 24 h after the bait was ingested was determined by transfer of females to clean vials 12 h after they were treated. The hourly rate of excretion of fipronil was also determined during the first 12 h after ingestion of bait by transfer of cockroaches hourly to clean vials and by subjecting of the old vial to LSC.

Origin of the Fipronil-Containing Residues

In the time course assays, and in freely ranging treated cockroaches, liquid excretions were apparent on many of the cockroaches, and some died (ventral side up) in a pool of liquid that accumulated under their wings. Yet, it is important to note that no fipronil was excreted before the onset of paralysis. To determine the origin of these excretions, bait-fed females were placed individually in a specially designed filter paper harness to collect excreted residues. Twenty day-2 females, starved for 24 h, were fed radioactive fipronil as before and held in plastic dishes where they developed symptoms of poisoning (3–5 h after ingestion) and became paralyzed. At this time they were placed dorsal side down onto three separate pieces of filter paper (No. 1 Qualitative; Whatman, Clifton, NJ), one directly under the head region, another under the thorax and anterior abdomen, and a third piece of filter paper under the three terminal tergites. Equal-sized aluminum foil pieces were placed under each filter paper to retain excretions on the papers. Each female was held in place with two additional pieces of filter paper secured with pins over its head and anal regions.

The harnesses and foils were subjected to LSC after 12, 24, and 48 h.

Attraction of Nymphs to Bait-Fed Adults

First instars readily die after contacting fipronil-poisoned adults even in the absence of cannibalism or adult feces (13). We hypothesized that nymphs might aggregate around fipronil-killed cockroaches and possibly ingest liquid excretions produced by moribund insects. The possibility that horizontal transmission of fipronil was mediated by transfer of oral or rectal excretions through contact or ingestion was therefore directly examined with time-lapse video recording of all visits to moribund cockroaches.

Fifteen first instars were placed in a 9 by 9-cm plastic dish containing a 3-cm^2 carton shelter in one corner and a water vial and a rat chow pellet in the corner opposite from the shelter. The nymphs were then allowed to acclimate to this dish for 12 h. Adult females, starved for one scotophase, were individually fed 1 mg of 0.03% Maxforce FC bait 4 h before the onset of the next scotophase in the absence of rat chow and water. Four h later, at the onset of paralysis, the females were placed dorsal side down in the corner opposite from the shelter housing the nymphs, 15 mm from the rat chow. To test whether cockroaches that ingested more bait could attract more nymphs, starved females were fed the bait to repletion and then assayed with first instars. A treatment block consisted of three dishes: one containing a female that ingested 1 mg bait, one in which the female ingested bait to repletion, and one containing a cyanide-killed control female. This block was videotaped simultaneously and replicated six times. Activity of the nymphs was monitored with an infrared-sensitive video camera (Panasonic WV-BL730; Secaucus, NJ) equipped with a 12.5- to 75-mm zoom lens and connected to a time-lapse video recorder (Panasonic AG-6740) providing about 2.5 images per second. Video records were analyzed frame-by-frame and nymph contacts with the rat chow pellet, water, and oral and anal regions of the female were tabulated without regard to the identity of each of the 15 nymphs. Visits to the mouth and its immediate vicinity, including the thorax, were categorized as visits to the oral region, and visits to the abdomen and the anus were categorized as visits to the rectal region. The experiment was designed so that the onset of nymphal activity (2 h into scotophase) coincided with the onset of fipronil excretions. Data representing 135 min of foraging activity commencing 2 h into the scotophase were used for statistical analysis. This design also precluded the confounding effect of inclusion of nymphs that showed signs of secondary fipronil poisoning.

This experimental design was also used to elucidate the effects of starvation in first instars on relative visitation to fipronil-killed adult females. Under field conditions, where food and water are often limiting or far away from the shelter, cockroaches that ingest fipronil bait and die within the shelter might be the only source of food and water readily available to nymphs. We hypothesized that visits to the dying cockroaches, and therefore secondary kill, would increase. Visits to fipronil-killed cockroaches in the absence of food and water were therefore evaluated by time-lapse video. The nymphs had continuous access to rat chow which was removed from the dishes and replaced with dying females just prior to the start of videotaping. Control experiments with cyanide-killed females were performed simultaneously. A treatment block, consisting of two dishes of fipronil-killed females and two dishes of cyanide-killed females, was replicated three times.

RESULTS

Time Course and Sources of Fipronil Excretion

Most (79%) of the eliminated radioactivity from [^{14}C]fipronil was excreted during the first 12 h after ingestion of the bait. Mean ^{14}C excretion during the first 12 h was 2483 $\pm$ 535 dpm, equivalent to 19 ng fipronil (n = 20) if we assume that no radiolabeled metabolites were excreted. Vials that harbored females for 24 h contained 3137 $\pm$ 498 dpm (24 ng fipronil), demonstrating that little fipronil was excreted after the first 12 h. This pattern of excretion was confirmed in an independent experiment in which excretion was quantified in two successive 12-h periods. On average, only 1080 ± 361 dpm (8 ng fipronil) was excreted between 12 and 24 h after ingestion. Hourly extracts of the vials revealed that no fipronil was excreted during the first 3 h after ingestion of the bait, a time during which none of the cockroaches exhibited symptoms of poisoning (Fig. 1). Fipronil excretion rose dramatically at approximately the time when paralytic symptoms first appeared, at 4–6 h, and then gradually declined. These results justified the delay in the collection of excretions in filter paper harnesses until the onset of paralysis.

In filter paper harnesses, >74% of the total radioactivity excreted from [^{14}C]fipronil-fed females was recovered from the oral region in the first 12 h (Fig. 2). The remaining fipronil was recovered from filter papers under the anal region, and no radioactivity was eliminated from the rest of the body (including the thorax and most of abdominal segments). Interestingly, excretion from the anal region, though minor, continued for up to 48 h (Fig. 2).

Validation of the Experimental Design

Because we examined oral and anal exudates, it was required that control cockroaches not excrete any liquids while handled or anesthetized. Yet, CO_2-anesthetized cockroaches regurgitated liquids, and cockroaches killed by freezing collected atmospheric moisture during freezing and thawing. In contrast, cockroaches killed with potassium cyanide vapors did not excrete liquids before they died. To validate that the latter females were neither toxic nor repellent, they were compared to freezer-killed cockroaches in bioassays with first instars.

Adult females were killed by freezing at −80°C (3 min) and then thawed at room temperature. Another group of females was killed by exposure to cyanide vapors (10 min) and then frozen and thawed, as above. At the onset of the scotophase, individual dead females were transferred to 9 by 9-cm dishes containing 15 first instars and assayed, as previously described. The video results showed that cyanide-killed frozen cockroaches were no more repellent or toxic to untreated nymphs than cockroaches killed by freezing alone. Adult females killed by either method were equally contacted by nymphs (Student's *t* test, $t = 0.200$, $n = 6$, $P = 0.845$). On average, first instars made 46.7 ± 17.0 visits to cyanide-killed females and 42.8 ± 8.8 visits to freezer-killed females during the 135-min observation period. Moreover, no nymphs died in any of the assays, even when rat chow was removed

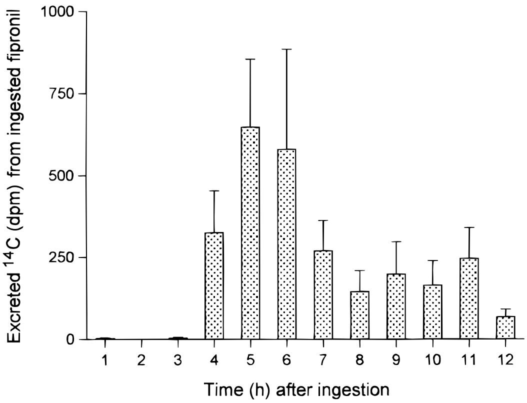

FIG. 1. Time course of ^{14}C excretion by adult female *B. germanica* fed [^{14}C]fipronil bait. Bars represent mean hourly dpm excreted per insect + SE ($n = 20$).

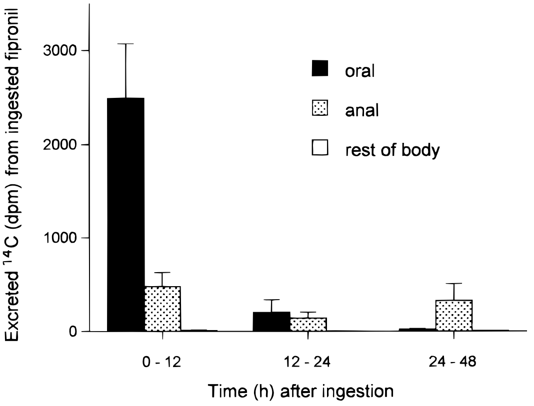

FIG. 2. Elimination of ^{14}C from oral, anal, and thoracic–abdominal (rest of body) regions of adult female *B. germanica* fed [^{14}C]fipronil bait. Bars represent mean dpm excreted per insect + SE ($n = 20$).

from the assay, and nymph contacts with cyanide-killed females increased 4.7-fold (218.7 ± 15.4 contacts in 135 min). We therefore conclude that a brief exposure of adult females to cyanide, followed by aeration, does not diminish their attractiveness or palatability to first instars, and cyanide-killed females are appropriate controls in these experiments.

Behavioral Response of Nymphs to Adult Excretions

Behavioral observations revealed that exudates from fipronil-fed cockroaches were highly attractive to first instars. We did not record the duration of contacts, nor did we distinguish between single and multiple visits by each of the 15 nymphs during the 135-min assays. In cages containing fipronil-killed females 38% of the first instar contacts were with the female, 44% with rat chow, and 18% with water (Fig. 3). In control cages, only 14% of the contacts were with dead cockroaches, whereas the majority were with rat chow (68%) and water (18%). Because nymph activity was not equivalent in all dishes, we normalized contacts with the females as a function of contacts with rat chow within each assay. The nymphs contacted fipronil-killed females and rat chow almost equally (0.9 ± 0.2 female contacts per contact with rat chow), whereas contacts with cyanide-killed females were 78% lower (0.2 ± 0.0 contacts per contact with rat chow). Although first instars were attracted significantly more to fipronil-killed females than to control females (Mann–Whitney *U* test, $Z = 2.322$, $n = 6$, $P = 0.020$) both groups equally visited the water vial (Fig. 3). Thus, it appears that fipronil-killed females drew nymphs away from the rat chow pellet rather than from the water source.

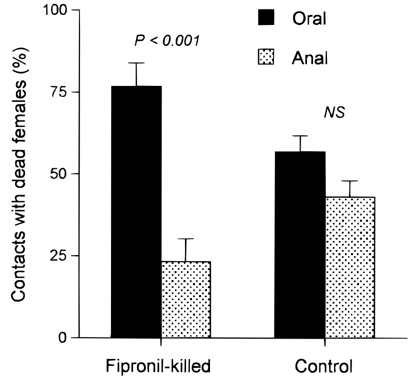

FIG. 4. Visits to the oral or anal regions of control or fipronil-killed females by first instars in choice assays (with rat chow and water present). Bars represent mean percentage of visits + SE of six replicates. Significant differences between means were determined by the *G* test.

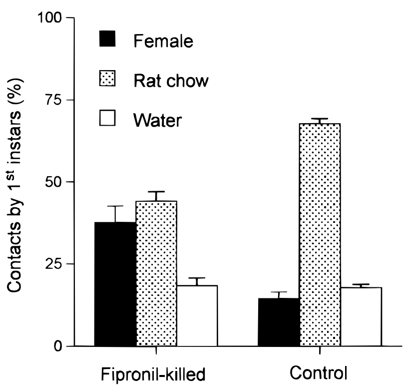

FIG. 3. Visits to control or fipronil-killed female, rat chow, and water by first instars in choice assays. Bars represent mean percentage + SE of total contacts within each cage (six replicates).

In cages containing fipronil-killed females, 77% of the contacts by first instars were with the oral region, the primary source of excreted fipronil (Fig. 4). The remaining 23% of the contacts were with the anal region, significantly less than with the oral region (*G* test, $G = 30.29$, $df = 1$, $P < 0.001$). In the control experiment, on the other hand, the number of contacts with the oral (57%) and anal (43%) regions were not significantly different (*G* test, $G = 1.91$, $df = 1$, $P > 0.05$). These results, showing a correlation among the origin of fipronil-containing excretions, the amount of fipronil excreted, and the patterns of visitation of first instars, suggest that nymphs are attracted to regurgitated bait. They further suggest that cockroaches that return to the shelter after feeding on fipronil bait might effectively attract conspecifics and transfer fipronil to them.

Females that fed on the bait *ad libitum* and presumably were capable of regurgitating a greater amount of bait did not attract any more nymphs than females fed 1 mg of bait (Mann–Whitney *U* test, $Z = 1.201$, $n = 6$, $P = 0.230$). However, first instars contacted fipronil-killed females 2.7-fold more when other food was not available, and >88% of the nymphs died in less than 12 h. When rat chow was available, fewer nymphs died (Mann–Whitney *U* test, $Z = 2.882$, $n = 6$, $P = 0.004$) and cumulative mortality did not exceed 58% in 12 h. This suggests that cockroaches that feed on a fipronil bait and die within the shelter might effectively transfer fipronil to conspecifics, especially to small nymphs that had not fed.

DISCUSSION

Emetophagy: Trophallaxic Dissemination of Emetogenic Insecticides

This study describes a new mechanism by which bait toxicants are dispersed within *B. germanica* aggregations. We define emetophagy as the ingestion of regurgitates that are induced by emetic chemicals. In this study, we describe this novel phenomenon with fipronil, but it is possible that other fast-acting, neurotoxic insecticides are also emetogenic. Fipronil is a potent neurotoxin that disrupts the central nervous system and overstimulates the insect's nerves and muscles. While some fast-acting neurotoxic insecticides disrupt water balance in insects by inducing diuresis (18–20), this effect was not clearly evident in *B. germanica*. Rather, acute emesis, a common symptom of insecticide poisoning (21–23) and an important mechanism in the voiding of gut contents with several insecticides, is elicited by fipronil in the German cockroach. Results of the behavioral experiments indicate that the regurgitated bait is attractive to other cockroaches and horizontal transfer of fipronil thus occurs when untreated cockroaches either contact or ingest the regurgitated bait.

Trophallaxis, "the exchange of alimentary liquid among colony members or guest organisms, either mutually or unilaterally," (24) is ubiquitous in social insects. The emetophagic exchange of bait that we describe in *B. germanica* shares many similarities with the trophallaxic exchange of bait in social insects. Both processes involve transfer of regurgitated food from one insect to another, and both facilitate the transfer of toxicants in aggregations of insects. Despite the obvious similarities, important differences exist between the two processes. First, cockroaches do not actively solicit bait regurgitate from other cockroaches, but rather opportunistically ingest whatever regurgitate becomes available. Second, in the German cockroach, ingestion of regurgitated food is facultative, in contrast to social insects, in which some castes cannot or do not forage and have an obligatory dependence on their nestmates for nutrition (also in parent–nestling food exchange in birds). Nevertheless, both obligatory trophallaxis in social insects and facultative emetophagy in cockroaches can play central roles not only in nutrient exchange, but also in insecticide flow among members of a colony or aggregation. Horizontal dissemination of insecticides through trophallaxis has been amply demonstrated in ants (25–27) and termites (28–31).

In cockroaches, as in social insects, exchange of insecticides by emetophagy resulted in delivery of toxicants to relatively more sedentary stadia. Results of our radiotracer experiments demonstrate that most of the excreted fipronil originates from the mouth. The excreted fipronil, mixed in an attractive and palatable regurgitate, is readily ingested by nymphs, especially in the absence of naturally occurring food. The most striking feature of this behavior is the positive correlation between the source of the excretions and the number of visits made by the nymphs to that body region. Our results show that the nymphs selectively aggregate and forage around the oral region of moribund cockroaches, the region from which most of the excretions emanate. Although we have not quantified the average duration of feeding on those excretions we frequently observed nymphs pausing for up to 5 min to feed at a single location.

Comparison of Slow- and Fast-Acting Insecticides

In social insects, where the primary objective is to deliver the insecticide to the queen(s), delayed-action insecticides have traditionally been favored to give the foraging workers ample time to deliver and share the toxicant with other members of the colony. Recently, however, fipronil, a fast-acting insecticide, was shown to be effectively disseminated by trophallaxis in colonies of the red imported fire ant (32) and the Argentine ant (33) when used in ultra-low-dose formulations. Furthermore, it has been suggested that, in ants, fipronil may increase the rate of trophallaxis, whereas delayed-action hydramethylnon may slow it down (31).

With cockroaches, the primary goal, of course, is to kill foraging insects and dissemination of the insecticide to the aggregation is a subsidiary objective. Necrophagy has been implicated in dissemination of insecticides in populations of *B. germanica* (9, 11) and *Blatta orientalis* L. (10). Gahlhoff *et al.* (9) reported 100% secondary mortality with fipronil when bait-fed dead nymphs were the only source of food available to caged untreated cockroaches; mortality with hydramethylnon did not exceed 65% even though more nymphs were consumed. Durier and Rivault (11), too, reported fipronil-induced secondary kill through necrophagy in the German cockroach, and secondary mortality was high even though few of the bait-fed nymphs were cannibalized by starved adults. Necrophagy thus occurs with both fast- and slow-acting insecticides.

Although more insecticide is excreted when cockroaches ingest slow-acting insecticides, both fast- and slow-acting insecticides can result in significant secondary kill, but through different mechanisms. Adult male cockroaches excreted approximately 4.5% of the fipronil that they ingested (12), primarily due to emesis. The remainder becomes sequestered within the dead cockroach, as fipronil kills cockroaches soon after ingestion. Secondary mortality can thus be effected by emetophagy, necrophagy, or both. In contrast to fipronil, studies with hydramethylnon showed that adult males excreted 22.3% of the total ingested hydramethylnon and other life stages excreted even more (range: 29.7% for fifth instars to 44.7% for nongravid females) (4). Since more than 99% of [^{14}C]hydramethylnon from bait-fed males is excreted in the feces, coprophagy appears to be the major mechanism mediating horizontal transfer of hydramethylnon (4), and necrophagy probably plays a lesser role.

As in social insects, the extent of secondary kill with either slow- or fast-acting insecticides should be profoundly affected by whether foragers return to the shelter and their subsequent interactions with other insects. Several studies highlight that cockroaches, especially neonates, prefer to utilize local food sources and minimize foraging when adequate food is present nearby. Kopanic and Schal (8) have shown that coprophagy in small nymphs more than doubled when the distance between food and aggregation was increased from 2 to 120 cm, and coprophagy was even greater in starved nymphs. Reierson and Rust (34) have shown that food consumption by cockroaches increased 2-fold when the food was placed inside or next to shelters rather than 45 cm away. Proper bait placement, too, has been shown to be critical for maximizing cockroach control (35–37). Two observations suggest that dissemination of ingested fipronil may be constrained in field populations. First, hyperactivity and convulsions in fipronil-fed cockroaches severely interfere with their ability to remain within the shelter and place the dying foragers at some distance from the shelter (13). Because the regurgitated fipronil is closely associated with the dying cockroaches, neonates within the shelter are less likely to contact the dying foragers. Second, the bioavailability of regurgitated fipronil diminishes greatly as the oral excretions dry (12). Nevertheless, those cockroaches that ingest fipronil and die within or near the shelter exude attractive and palatable oral regurgitates that facilitate secondary kill. The results of our videotaping experiments show that nymphs contacted fipronil-killed females 2.7-fold more when other food was not available. Mortality in the nymphs was $>$88%, demonstrating that cockroaches returning to the shelter may effectively disseminate fipronil to small nymphs. Similarly, le Patourel (10) showed that secondary mortality with fipronil in *B. orientalis* increased significantly in starved nymphs.

Slow-acting insecticides (e.g., hydramethylnon) are expected to be disseminated mainly in the feces. Indeed, 51% of the hydramethylnon in the feces is the unmetabolized parent compound (4), and therefore hydramethylnon-containing feces should retain their toxicity for a substantial amount of time. Moreover, the feces and bodies of hydramethylnon-killed foragers are distributed within and near the shelter (13), making both more readily available to other insects.

The level of secondary kill that can be attained with these two very different insecticides will depend on many factors, such as concentration of the active ingredient (AI) in the bait, stability of the AI in the insect's digestive tract, relative toxicity of the AI, amount of the AI excreted, amount of the AI bioavailable after excretion, mechanism of dispersion, and possibly other factors. Since interactions among all of these factors have not yet been explored, it is impossible at this time to conclusively state which of the two insecticides is more effectively dispersed in aggregations of cockroaches. The potential of fipronil and hydramethylnon to cause secondary kill can be estimated only through detailed field studies. What remains to be determined is the importance of the indirect uptake of fipronil, and other bait toxicants, for practical pest control. The potential for horizontal transfer to occur under field conditions needs to be investigated in studies that could not only readily differentiate between primary and secondary mortality but could also, by measuring secondary kill, assess the extent to which bait-fed cockroaches that return to the shelter facilitate the dissemination of the insecticide.

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REFERENCES

1. R. Metzger, Behavior, *in* "Understanding and Controlling the German Cockroach" (M. K. Rust, J. M. Owens, and D. A. Reierson, Eds.), pp. 49–76, Oxford Univ. Press, New York, 1995.
2. R. J. Kopanic, Jr., G. L. Holbrook, V. Sevala, and C. Schal, An adaptive benefit of facultative coprophagy in the German cockroach, *Blattella germanica* (L.), *Ecol. Entomol.* **26,** 154 (2001).
3. C. Schal, G. L. Holbrook, J. A. S. Bachmann, and V. L. Sevala, Reproductive biology of the German cockroach, *Blattella germanica*: Juvenile hormone as a pleiotropic master regulator, *Arch. Insect Biochem. Physiol.* **34,** 405 (1997).
4. J. Silverman, G. I. Vitale, and T. J. Shapas, Hydramethylnon uptake by *Blattella germanica* (Orthoptera: Blattellidae) by coprophagy, *J. Econ. Entomol.* **84,** 176 (1991).
5. H. S. J. Ree and I. Lee, Laboratory test on secondary killing effect of hydramethylnon by coprophagous uptake of *Blattella germanica, Jpn. J. Sanit. Zool.* **4,** 397 (1995).
6. W. Kaakeh, B. L. Reid, and G. W. Bennett, Toxicity of fipronil to German and American cockroaches, *Entomol. Exp. Appl.* **84,** 229 (1997).
7. R. J. Jr. Kopanic and C. Schal, Relative significance of direct ingestion and adult-mediated translocation of bait to German cockroach nymphs (Dictyoptera: Blattellidae), *J. Econ. Entomol.* **90,** 1073 (1997).
8. R. J. Kopanic, Jr., and C. Schal, Coprophagy facilitates horizontal transmission of bait among cockroaches (Dictyoptera: Blattellidae), *Environ. Entomol.* **28,** 431 (1999).
9. J. E. Gahlhoff, Jr., D. M. Miller, and P. G. Koehler, Secondary kill of adult male German cockroaches (Dictyoptera: Blattellidae) via cannibalism of nymphs fed toxic baits, *J. Econ. Entomol.* **92,** 1133 (1999).
10. G. N. J. le Patourel, Secondary transmission of fipronil toxicity between Oriental cockroaches *Blatta orientalis* L. in arenas, *Pest Manag. Sci.* **56,** 732 (2000).
11. V. Durier and C. Rivault, Secondary transmission of toxic baits in German cockroach (Dictyoptera: Blattellidae), *J. Econ. Entomol.* **93,** 434 (2000).
12. G. Buczkowski and C. Schal, Method of insecticide delivery affects horizontal transfer of fipronil in the German cockroach (Dictyoptera: Blattellidae), *J. Econ. Entomol.* **94,** 680 (2001).
13. G. Buczkowski, R. J. Kopanic, Jr., and C. Schal, Transfer of ingested insecticides among cockroaches: Effects of active ingredient, bait formulation, and assay procedures, *J. Econ. Entomol.* **94,** 1229–1236 (2001).
14. S. M. Valles, C. A. Strong, and P. G. Koehler, Inter- and intra-instar food consumption in the German cockroach, *Blattella germanica, Entomol. Exp. Appl.* **79,** 171 (1996).
15. H. P. Young and C. Schal, Cuticular hydrocarbon synthesis in relation to feeding and developmental stage in nymphs of *Blattella germanica* (Dictyoptera: Blattellidae), *Ann. Entomol. Soc. Am.* **90,** 656 (1997).
16. D. G. Cochran, Food and water consumption during

the reproductive cycle of female German cockroaches, *Entomol. Exp. Appl.* **3,** 51 (1983).

17. R. Hamilton and C. Schal, Effects of dietary protein levels on reproduction and food consumption in the German cockroach (Dictyoptera: Blattellidae), *Ann. Entomol. Soc. Am.* **81,** 969 (1988).
18. S. H. P Maddrell and J. E. Casida, Mechanism of insecticide-induced diuresis in *Rhodnius, Nature* **231,** 55 (1971).
19. S. H. P Maddrell and S. E. Reynolds, Release of hormones in insects after poisoning with insecticides, *Nature* **236,** 404 (1972).
20. Y. A. Abdel-Aal and M. H. Hussein, Cypermethrin-induced diuresis in *Bombyx mori* L. larvae, *J. Appl. Entomol.* **93,** 489 (1982).
21. L. Lagadic, W. Leicht, M. G. Ford, D. W. Salt, and R. Greenwood, Pharmacokinetics of cyfluthrin in *Spodoptera littoralis* (Boisd.) I. *In vivo* distribution and elimination of [^{14}C]cyfluthrin in susceptible and pyrethroid-resistant larvae, *Pestic. Biochem. Physiol.* **45,** 105 (1993).
22. J. A. Wiles and P. C. Jepson, The dietary toxicity of deltamethrin to the carabid, *Nebria brevicollis* (F.), *Pestic. Sci.* **38,** 329 (1993).
23. P. K. Michaelides and D. J. Wright, Insecticide penetration and symptomology studies on larvae of *Diabrotica indecimpunctata howardi* (Barber), *Pestic. Sci.* **49,** 353 (1997).
24. B. Hölldobler and E. O. Wilson, "The Ants," Belknap Press of Harvard Univ. Press, Cambridge, MA, 1990.
25. T. H Su, J. W. Beardsley, and F. L. McEwen, AC 217,300, a promising new insecticide for use in baits for control of the bigheaded ant in pineapple, *J. Econ. Entomol.* **73,** 755 (1980).
26. W. A Banks, H. L. Collins, D. F. Williams, C. E. Stringer, C. S. Lofgren, D. P. Harlan, and C. L. Mangum, Field trials with AC 217,300, a new amidinohydrazone bait toxicant for control of the red imported fire ant, *Southwest. Entomol.* **6,** 158 (1981).
27. J. H. Klotz and B. L. Reid, Oral toxicity of chlordane, hydramethylnon, and imidacloprid to free-foraging workers of *Camponotus pennsylvanicus* (Hymenoptera: Formicidae), *J. Econ. Entomol.* **86,** 1730 (1993).
28. N. Y. Su, M. Tamashiro, J. R. Yates, and M. I. Haverty, Effect of behavior on the evaluation of insecticides for prevention of or remedial control of the Formosan subterranean termite, *J. Econ. Entomol.* **75,** 188 (1982).
29. J. K Grace, Response of eastern and Formosan subterranean termites (Isoptera: Rhinotermitidae) to borate dust and soil treatments, *J. Econ. Entomol.* **84,** 1753 (1991).
30. U. Schoknecht, D. Rudolph, and H. Hertel, Termite control with microencapsulated permethrin, *Pestic. Sci.* **40,** 49 (1994).
31. A. Peppuy, A. Robert, J. P. Delbecque, J. L. Leca, C. Rouland, and C. Bordereau, Efficacy of hexaflumuron against the fungus-growing termite *Pseudacanthotermes spiniger* (Sjosted) (Isoptera, Macrotermitinae), *Pestic. Sci.* **54,** 22 (1998).
32. H. L. Collins and A. M. A. Callcott, Fipronil: An ultra-low-dose bait toxicant for control of red imported fire ant (Hymenoptera: Formicidae), *Fl. Entomol.* **81,** 407 (1998).
33. L. M. Hooper, "The Nutritional Ecology and Effects of Toxicants on Colonies of Argentine ants, *Linepithema humile* (Mayr)," Ph.D. dissertation, University of California, Riverside, CA, 1998.
34. D. A. Reierson and M. K. Rust, Insecticidal baits and repellency in relation to control of the German cockroach, *Blattella germanica* (L.), *Pest Manag.* **2,** 26 (1984).
35. D. A. Reierson, M. K. Rust, A. M. Van Dyke, and A. G. Appel, Control of German cockroaches with amidinohydrazone bait, 1982, *Insectic. Acar. Tests* **8,** 54 (1983).
36. M. H. Ross, Laboratory studies on the response of German cockroaches (Dictyoptera: Blattellidae) to an abamectin gel bait, *J. Econ. Entomol.* **86,** 767 (1993).
37. J. Silverman, Adult German cockroach (Orthoptera: Blattellidae) feeding and drinking behavior as a function of density and harborage-to-resource distance, *Environ. Entomol.* **15,** 198 (1986).