

FACTORS AFFECTING SECONDARY KILL OF THE GERMAN COCKROACH (DICTYOPTERA: BLATTELLIDAE) BY GEL BAITS

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Abstract Secondary kill of the German cockroach, *Blattella germanica* (L.), by baits was reported to increase the overall control efficacy of bait products. However, most studies have been based on laboratory strains and small nymphs. We compared the secondary kill of four cockroach gel baits against various developmental stages of a laboratory (Jwax) and a field (Dorie) strain *B. germanica*. The four baits were: 0.35% acetamiprid (Transport), 0.01% fipronil (Maxforce FC), 2.15% hydramethylnon (Maxforce), and 0.6% indoxacarb (Advion). In addition, the secondary kill by acetamiprid, hydramethylnon, and indoxacarb was evaluated against mixed-stage cockroach populations. All baits exhibited secondary kill against various developmental stages of *B. germanica*. The levels of secondary mortality decreased from 100% in the first instars to as low as 12.1% in adult males. The field strain was much less susceptible than the laboratory strain, with only 9.2-16.6% secondary mortality among the 3rd-4th instars. Acetamiprid caused significantly lower secondary mortality of the laboratory strain first instars than fipronil, hydramethylnon, and indoxacarb. In an experiment evaluating direct and secondary kill against mixed-stage populations (100 total per experimental arena), the direct kill by acetamiprid, hydramethylnon, and indoxacarb was 40.0, 74.0, and 98.5%, respectively. Only indoxacarb caused significant secondary kill at 0.985 donor/recipient ratio. Acetamiprid and hydramethylnon did not cause detectable secondary kill at donor/recipient ratios of 0.40 and 0.74, respectively. We conclude the level of secondary kill by baits was highly influenced by cockroach developmental stage, strain, and donor/recipient ratio.

Key Words *Blattella germanica*, gel bait, secondary kill, toxicant transfer

INTRODUCTION

Bait (gel bait or containerized solid bait) is currently the primary formulation used for managing the German cockroach, *Blattella germanica* (L.), in the United States. Bait has the advantage over liquid or dust formulations because baiting requires shorter service time, has shown increased efficacy, and has reduced environmental contamination. Although baits are usually more expensive than liquid or dust insecticides in terms of materials cost for control, their benefits outweigh the higher product cost. It is recognized that the effectiveness of a bait is mainly determined by: 1) palatability of the bait matrix; and 2) toxicity and non-repellency of the active ingredient used in the bait (Reiersen 1995). In addition, the ability to cause secondary kill is reported as a factor contributing to the overall efficacy of a bait product (Silverman et al., 1991; Kopanic and Schal, 1997; Gahlhoff et al., 1999; LePatourel, 2000; Buczkowski and Schal, 2001a). Secondary kill is the mortality of unexposed cockroaches, which can occur through contact with or feeding on poisoned cockroaches (cannibalism, necrophagy), excretions produced from poisoned cockroaches (coprophagy), or oral secretions passed from poisoned cockroaches (emetaphagy).

Silverman et al (1991) first reported the secondary kill of a hydramethylnon-based bait. In this study, a lethal dose of hydramethylnon was detected in the feces of *B. germanica* that were consuming bait. The mechanism of transfer was attributed to coprophagy. The slow-acting nature of hydramethylnon was recognized as being important for its effect on un-exposed cockroaches. Hydramethylnon exerted its greatest effect on early instars through coprophagy.

The levels of secondary kill varied with the active ingredients and formulations (Gahlhoff et al., 1999; Buczkowski and Schal, 2001b). Both hydramethylnon and fipronil baits were much more effective than

abamectin in secondary kill. Gel formulations resulted in greater secondary mortality than powder or solid bait formulations (Buczowski and Schal, 2001b). Smith et al (2002) compared the effect of secondary kill of 0.5% noviflumuron and 2.15% hydramethylnon (Maxforce) gel bait against *B. germanica* first instars. The nymphs were provided with dog food and feces excreted from poisoned cockroaches. After 14 d exposure, feces containing noviflumuron and hydramethylnon caused 65 and 83% control mortality, respectively.

Two newer baits, 0.6% indoxacarb (Advion) and 0.34% acetamiprid were registered in the U.S. since 2005. Both were advertised by manufactures as having secondary kill property. Unlike hydramethylnon, indoxacarb is a very fast acting compound which causes 100% knockdown within a few hours to 24 hours after ingestion in our studies (unpublished data). We also observed that indoxacarb-poisoned cockroaches produced a distinct liquid excretion at the end of the cockroach abdomen within 24 hours of bait ingestion. The knocked-down cockroaches were often found “glued” to the experiment arenas by the excretion until eventual death. Exposure to the excretion caused significant mortality to *B. germanica* in our preliminary studies.

Although secondary kill by cockroach baits was reported in various studies, its relative importance in overall cockroach mortality is not clear. Previous experiments evaluated secondary kill of laboratory cockroach strains, which are much more susceptible to insecticides than field strains. It would be more meaningful for product users to know the degree of secondary kill against field strains. The objectives of this study were to: 1) compare the effectiveness of secondary kill of four gel baits against various stages of laboratory and field strain cockroaches; and (2) evaluate the secondary kill against mixed-stage German cockroach populations by three gel baits.

MATERIALS AND METHODS

Insects

Two *B. germanica* strains, a laboratory (Jwax) and a field (Dorie) strain, were included in the study. Jwax was maintained in the laboratory for > 30 years. This strain was reared on Teklad rodent chow (Harlan Teklad, Madison, WI, USA) *ad libitum*. The field strain was collected in apartments in 2003 (Wang et al. 2004). This strain was reared on rodent chow, creamy peanut butter, and grape jelly *ad libitum*. The behavioral and physiological resistance levels of this strain were reported by Wang et al. (2004). The field strain has low levels of physiological resistance to fipronil and abamectin. It has significantly less feeding response to gel baits than the laboratory strain. Our preliminary studies indicated that cockroaches fed on single diet for extended period will consume more bait than those fed on mixed diet. Therefore, we maintained the field strain on mixed diet in order to maintain the cockroaches' foraging characteristics. Levels of secondary mortality are expected to be affected by the different consumption levels and physiological differences between laboratory and field cockroach strains. Including a laboratory strain in this study was intended to detect changes in secondary kill among developmental stages because laboratory strain is more likely to exhibit secondary mortality from cockroach bait than field strains. Whereas, including a field strain will allow us to compare differences in secondary kill between laboratory and field strains. The cockroaches were maintained in 40.5 × 28.0 × 14.5 cm plastic boxes in walk-in environmental chambers at 26°C, 60% RH, and 12:12 h [L:D] photoperiod.

Baits

Four cockroach gel baits were studied. They were: 0.35% acetamiprid (Transport roach bait, FMC Corporation, Philadelphia, PA), 0.6% indoxacarb (Advion cockroach gel bait, E. I. duPont de Nemours and Company, Wilmington, DE), 0.01% fipronil (Maxforce FC roach killer bait gel, Bayer Environmental Science, Raleigh, NC), and 2.15% hydramethylnon (Maxforce roach bait gel, Bayer Environmental Science, Raleigh, NC). These baits were selected because they represented a range of resultant knockdown activity from hours to days. All these baits were claimed to cause secondary mortality to *B. germanica* by manufactures. Yet, their relative effectiveness was unknown. The baits were purchased from a local distributor.

Experimental Design

Small-box experiment. One day before the treatment, five groups of 45-50 adult male *B. germanica* were transferred from a rearing container to five boxes (L × W × H: 18.7 × 13.3 × 9.5 cm). These cockroaches

were called “donors”. On the same date, 25 boxes were prepared. Twenty *B. germanica* were transferred to each box. These cockroaches were called “recipients”. The cockroaches were provided with a cardboard tent harborage and a water vial. The upper inner walls of the boxes were covered with a thin layer of petroleum jelly and mineral oil (1:2) to prevent cockroaches from escaping. After approximately 20 hours and immediately before the dark cycle, 2 g 1-d-old cockroach gel bait was added to each donor box. We used 1-d-old bait because under field conditions, bait placements are usually discovered by foraging cockroaches after several hours to a few days. Each of the four donor boxes received different bait. The 5th donor box received rodent chow as control. The donors were allowed to feed on bait or rodent chow for 2 h. Then, 10 donors were transferred to each recipient box. Some donors from the 0.6% indoxacarb, 0.01% fipronil, and 0.35% acetamiprid treatments showed symptoms of poisoning at the time of transfer, indicating very fast feeding activity and high susceptibility to these fast-acting compounds. Each of the donor boxes provided donors for five recipient boxes. Five experiments were conducted. In all experiments, the donors were Jwax adult males. The recipients in the five experiments were laboratory strain first instars, 3rd — 4th instars, and adult males; field strain first instars and 3rd — 4th instars (Table 1a). In the experiment where adult males were recipients, 1/3 of the donors’ left antennae were cut off to distinguish between the donors from the recipients. No abnormal feeding behavior or mortality was observed in the control donor cockroaches. A piece of rodent chow was added to each box as food source. Mortality of both donors and recipients were recorded daily until 10 d. Dead recipients were removed daily. The experiments were conducted in a walk-in chamber at 26°C with a 12:12 h L/D photoperiod.

Arena experiment. This experiment was designed to evaluate the direct and secondary mortality of mixed-stage cockroach populations (100 cockroaches). Bioassay arenas 1.0 × 1.0 × 0.25 m (base area 1.0 m²) were constructed from Plexiglas™ sheets (walls) and 2 cm thick particle board (floor) with a white painted surface. A thin layer of petrolatum and mineral oil (1:2) was applied to the arena walls to prevent cockroaches from escaping. Each arena had four cardboard tent harborages located at the center, two water vials at opposite corners, and one piece of rodent chow in one corner. A hundred field strain cockroaches with the following proportions: 15 males, 15 non-gravid females, 35 4th-5th instar nymphs, and 35 2nd-3rd instar nymphs were released into each arena. After 1 d, two plastic lids (4.3 cm diameter) with 1 d old gel bait (≈ 1 g/each) were placed at opposite corners of each arena. The baits included 0.35% acetamiprid, 2.15% hydramethylnon, and 0.6% indoxacarb. The arena experiment treatments represented three compounds with various knockdown and secondary kill properties. Hydramethylnon was a slow-acting compound. Acetamiprid had lower secondary kill than hydramethylnon and indoxacarb in small box assays. We did not include 0.01% fipronil in this experiment because of the limited number of experimental arenas and the fact that fipronil bait had similar level of secondary kill potential as indoxacarb in small box assays. No bait was placed in the control arenas. Each bait treatment was replicated four times.

After allowing the cockroaches to forage in the arenas for 2 d, the dead cockroaches were removed, sealed in plastic bags, and stored at 4°C. The purpose was to prevent from other healthy cockroaches feeding on the poisoned cockroaches and to maintain freshness of the bait killed cockroaches. Additional cockroaches died at the end of the 5 d period. The cadavers were left *in situ*. The baits and live cockroaches were removed from the arenas. We did not extend the period beyond 5 d because the poisoned cockroaches may become significantly less palatable to the recipients that will be released. The dead cockroaches saved in the refrigerator were placed back into each arena and spread randomly within the arenas. A new group of 100 field strain cockroaches with the same age structure as released earlier was introduced into each arena. They were exposed to the dead cockroaches, cockroach cast skins, cockroach excretions, and feces from the previous group of cockroaches. Surviving cockroaches were counted at 7 d. The experiment was conducted in a room at 27°C with a 12:12 h L/D photoperiod.

Data Analysis

The corrected mortality was calculated following Abbott (1925). Mortality data was transformed to arcsine of the square root and analyzed by ANOVA (SAS Institute 2001). Mean mortalities among treatments were separated by Tukey’s test at P = 0.05. Means between treatments and the control were separated with Dunnett test at P = 0.10.

RESULTS

Effects of Active Ingredient, Strain, and Stage on Secondary Mortality

The cumulative donor mortalities varied among the experiments (Table 1a). This might have been due to the very short period (2 h) when bait was provided. There were no significant differences in donor mortalities among baits, except that lower donor mortality occurred by acetamiprid treatment in one experiment. Mortalities in the untreated control were between 4-15%. Exposure to poisoned donors, their feces, and excretions caused detectable secondary mortalities to all stages of laboratory and field strain cockroaches (Table 1b). Mortalities in the untreated control were between 1- 17%. Feeding of dead cockroaches was observed in only three test boxes. Thus, necrophagy was not a major mechanism that was involved in the secondary mortalities. Acetamiprid caused significantly lower secondary kill against laboratory first instars compared with fipronil, hydramethylnon, and indoxacarb.

Table 1. Direct and secondary kill of *Blattella germanica* by gel baits in small box experiments. **A.** Direct mortality of laboratory strain adult males (donors).

Experiment		Cumulative donor mortality at 10 d (Mean ± SEM)*				ANOVA
Strain	Stage	Hydra-methylnon	Fipronil	Indoxacarb	Acetamiprid	
Lab strain	First instars	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	NA**
	3 rd — 4 th instars	100 ± 0.0a	95.3 ± 2.9a	95.6 ± 2.7a	65.3 ± 3.2b	F = 19.8; df = 3, 16; P < 0.001
	Adult males	86.9 ± 4.2a	87.8 ± 7.3a	86.7 ± 6.5a	82.7 ± 6.8a	F = 0.16; df = 3, 16; P < 0.92
Field strain	First instars	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	NA
	3 rd — 4 th instars	100 ± 0.0a	97.8 ± 2.2a	95.3 ± 2.9a	95.6 ± 2.7a	F = 0.93; df = 3, 16; P < 0.45

B. Secondary mortality after exposure to gel bait fed cockroaches

Cockroaches		Cumulative recipient mortality at 10 d (Mean ± SEM)*				ANOVA
Strain	Stage	Hydrame-thylnon	Fipronil	Indox-acarb	Acetamiprid	
Lab strain	First instars	98.9 ± 1.1a	100 ± 0.0a	100 ± 0.0a	66.3 ± 8.8b	F = 31.2; df = 3, 16; P < 0.001
	3 rd — 4 th Instars	55.4 ± 7.7a	31.6 ± 6.5a	47.5 ± 11.8a	32.1 ± 7.8a	F = 1.8; df = 3, 16; P = 0.20
	Adult males	12.1 ± 2.5a	20.3 ± 2.4ab	37.2 ± 7.2b	14.2 ± 1.9ac	F = 8.0; df = 3, 16; P = 0.002
Field strain	First instars	38.9 ± 5.0ab	64.4 ± 6.2a	56.7 ± 9.4ab	34.4 ± 2.1b	F = 4.9; df = 3, 16; P < 0.01
	3 rd — 4 th instars	10.2 ± 5.3a	16.6 ± 3.5a	12.1 ± 4.9a	9.2 ± 2.5a	F = 1.4; df = 3, 14; P = 0.28

Mean mortalities were corrected (Abbott, 1925). Means followed by different letters within the same row are significantly different (Tukey's test, P < 0.05). ** NA: Not available due to 0 variances.

The field strain was much less susceptible to secondary kill than the laboratory strain (Table 1 b). Among the four baits, secondary mortality of the field strain first instars was 31.9 — 60.0% lower than that of the laboratory strain. The secondary mortality of the field strain 3rd-4th instars was at least 15.0 — 45.2% lower than that of the laboratory strain. There was a clear inverse relationship between the cockroach developmental stage and the secondary mortality. Both laboratory and field strain first instars were much less susceptible to secondary kill than 3rd-4th instars. The adult males had the least secondary mortalities. The different levels of direct kill affected the donor/recipient ratios across the experiments. However, the much lower secondary mortality of adult males than 1st instars from hydramethylnon indicates the lowered donor/recipient ratios were not the main factor leading to the lower secondary mortalities in adult male recipients.

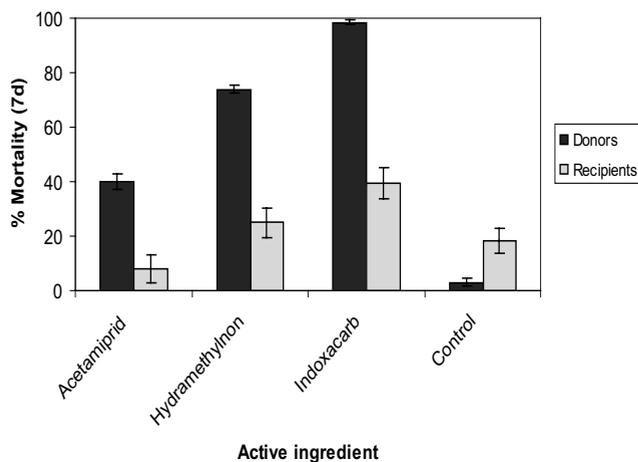


Figure 1. Direct and secondary kill of field strain *Blattella germanica* populations by three gel baits in 1 × 1 m arenas. Mean mortality of 100 mixed-stage cockroaches. Donors = solid; recipients = hatched.

Effect of Secondary Kill on Field Strain in Arena Experiment

Direct mortality (mean ± SEM) at 5 d by acetamiprid, hydramethylnon, and indoxacarb baits were 40.0 ± 2.6, 74.0 ± 1.5, and 98.5 ± 0.6%, respectively (Fig. 1). The mortality in the control arenas was 3.3 ± 1.3%. The level of direct kill by the four baits was: Advion > Maxforce > Transport (F = 233.6; df = 3, 12; P < 0.001).

Secondary mortality from exposure to indoxacarb, hydramethylnon, and acetamiprid killed cockroaches, and cockroach feces, and excretions were: 39.5 ± 5.8, 25.0 ± 5.5, and 8.0 ± 4.9%, respectively. The mean mortality in the control arenas was 18.3 ± 4.6%. Only indoxacarb bait caused detectable secondary kill against *B. germanica* populations (ANOVA: F = 4.8; df = 3, 10; P = 0.03; Dunnett test, P = 0.07). The corrected secondary mortality from indoxacarb bait was 26.0%.

DISCUSSION

All of the evaluated baits had the potential to cause secondary mortalities. Similar results were reported by Buczkowski et al. (2001), where all tested baits caused secondary mortality to *B. germanica* nymphs. The first instars exhibited the greatest secondary mortality, which might be associated with first instars' small size and feeding behavior. Because all donors were adult males and the number of recipients were equal across the experiments, small nymphs had opportunity to access greater amount of active ingredient based on their body weight. Small nymphs also were suggested having greater coprophagous behavior (Silverman et al., 1991). Thus, small nymphs were more likely to pick up a lethal dose of toxin through contact and feeding than larger nymphs or adults.

There was a clear inverse relationship between cockroach stages and secondary mortality. For example, secondary mortalities of the laboratory strain first instars, 3rd-4th instars, and adult males from uptake of hydramethylnon were 98.8, 55.4, and 12.1%, respectively. Although we did not conduct experiments against field strain adult cockroaches, we can safely expect that the secondary mortality would be even lower than

that found in the 3rd — 4th instars. The trend was opposite to that reported from Silverman et al (1991), where adult male mortality was significantly higher than nymphs after exposing to poisoned cockroaches. Silverman et al. (1991) tested mixed stages cockroaches. Whether the use of single or mixed stages has any effect on stage-specific susceptibility is not clear.

The field strain cockroaches were much less susceptible to secondary kill than the laboratory strain cockroaches. Two factors might have contributed to the lower secondary kill in field strain cockroaches: less bait consumption and greater tolerance to the active ingredients. Based on previous studies (Wang et al., 2004), the field strain consume significantly less bait than the laboratory strain. In addition, it is very possible that field strain cockroaches were more tolerant to the tested compounds than the laboratory strain due to multiple insecticide applications in apartments where the field strain cockroaches were collected.

Larger cockroach groups often exhibit slower and decreased mortality from bait treatments than smaller groups. Therefore, we evaluated secondary kill of mixed-stage cockroaches in 1 × 1 m arenas. The population study resulted in very different levels of direct kill (40 - 98.5%). As a result, the donor/recipient ratios were unequal in the subsequent experiment evaluating the secondary kill. The lower level of direct kill by acetamiprid bait was due to its inherent lower toxicity than hydramethylnon and indoxacarb baits, which was verified in additional tests of smaller groups (unpublished data). The lower level of direct kill by hydramethylnon may partially be due to its slower action which may require > 5 d to exhibit its full potential against mixed *B. germanica* populations. The actual donor/recipient ratios in the arena experiment were: acetamiprid - 0.40, hydramethylnon - 0.74, and indoxacarb - 0.985. At these ratios, both acetamiprid and hydramethylnon baits did not exhibit detectable level of secondary kill. Indoxacarb had significant secondary kill, indicating that secondary kill may contribute to the total mortality only when donor/recipient ratio reaches certain levels. Further studies on the relationship between the level of secondary mortality and donor/recipient ratios will help understand the role of secondary kill on the overall efficacy of cockroach baits.

In field environments, poisoned cockroaches and their excretions may not be readily accessible to other cockroaches due to more complex environments than that of the laboratory testing devices. The conditions of the poisoned cockroaches, their feces and excretions may be affected by the environmental conditions and human activities. The opportunity for food choice due to low levels of sanitation reduces the probability for cockroaches to feed on the bait-killed cockroaches or their excretions. In addition, field cockroaches may not eat as much bait as those under laboratory conditions because of the usually more diverse food sources, which dilutes the active ingredient concentration found in donor's cadavers, feces, or excretion. Therefore, the role of secondary kill may be much lower than that tested under laboratory conditions, especially when primary mortality is low. Thorough application of bait is important to provide direct access to maximum numbers of cockroaches in the environment.

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