

Effect of Fipronil on Bait Formulation-Based Aversion in the German Cockroach (Dictyoptera: Blattellidae)

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ABSTRACT We examined the effects of 3 active ingredients in baits containing glucose on a behaviorally resistant (glucose-averse, T-164) strain of *Blattella germanica* L. Baits with glucose and hydramethylnon or chlorpyrifos killed few T-164 individuals; however, fipronil-glucose baits killed 60% of the cockroaches by day 14. Although ingestion levels were low, fipronil-glucose baits were consumed more than hydramethylnon or chlorpyrifos glucose baits by T-164 cockroaches. Fipronil bait was 17 and 29 times more toxic to the T-164 strain than hydramethylnon and chlorpyrifos, respectively. Despite aversion to a key bait component, low-level bait consumption of a highly active toxicant such as fipronil may to some degree override bait inert-based behavioral resistance mechanisms.

KEY WORDS *Blattella germanica*, cockroaches, fipronil, behavioral resistance, glucose aversion

INSECTICIDAL BAITS ARE an important component in cockroach control programs because newer, relatively nonrepellent, active ingredients and improved formulations have made baits very efficacious. However, as is true for any insecticide formulation, widespread bait use increases the potential for resistance development, evidenced by reports of physiological and behavioral resistance to bait toxicants (Schal 1992, Ross 1998) and behavioral resistance to a bait inert ingredient (Silverman and Bieman 1993). Fipronil [\pm 5-amino-1-(2,6-dichloro- α,α,α -trifluoro-*p*-tolyl)-4-trifluoromethylsulfinyl-pyrazole-3-carbonitrile], a new insecticide with broad activity against several insect pests (Colliot et al. 1992), is highly toxic to cockroaches (Scott and Wen 1997). Kaakeh et al. (1997) report that fipronil is more toxic than chlorpyrifos or hydramethylnon, and that fipronil bait is not repellent and provides high cockroach mortality.

The T-164 and some other strains of the German cockroach, *Blattella germanica* (L.), reject glucose; consequently, hydramethylnon baits formulated with glucose as a feeding stimulant were not effective against these strains (Silverman and Bieman 1993, Silverman and Ross 1994). Although hydramethylnon is relatively nonrepellent (Appel 1990), it is also considerably less toxic than fipronil to cockroaches (Hollingshaus and Little 1984, Kaakeh et al. 1997). Chlorpyrifos is more toxic than hydramethylnon although less toxic than fipronil in nonresistant cockroach strains (Koehler and Patterson 1986) and is somewhat repellent in bait formulations (Appel 1990). Behavioral resistance to a bait inert can render a bait ineffective if a sublethal dose of active ingredient is in-

gested. The aim of the current study was to determine whether the relatively high toxicity and nonrepellent characteristics of fipronil could override glucose aversion in T-164 *B. germanica*. We suggest that the effectiveness of fipronil against this strain can be generalized to other cockroach strains displaying aversions to other bait inert ingredients such as surfactants, antimicrobials, binders, and food ingredients.

Materials and Methods

The strains of *B. germanica* used in these studies were Orlando normal and T-164. Orlando normal is a longstanding insecticide-susceptible strain with no known prior exposure to insecticides. T-164 glucose-averse (*Glu/Glu*) cockroaches were collected from a Florida apartment in 1991 (Silverman and Bieman 1993). Glucose aversion has been maintained by rearing the T-164 strain in the continuous presence of a bait containing 11.8% glucose and 2% hydramethylnon. Both cockroach strains were provided water and dog chow (Ralston Purina, St. Louis, MO) ad libitum. Environmental conditions for cockroach rearing and subsequent experiments were $27 \pm 2^\circ\text{C}$, $50 \pm 10\%$ RH, and a photoperiod of 12:12 (L:D) h.

A single bait matrix was used in all experiments. This matrix was formulated with either 0.03% fipronil, 2% hydramethylnon, or 0.5% chlorpyrifos (levels similar to those used in commercial products), and a fructose corn syrup (fructose bait) or fructose (55%) and glucose (42%) corn syrup (glucose bait).

Bait Performance. Baits were evaluated in plastic containers (45 by 30 by 20 cm), each provisioned with a cardboard harborage preconditioned for 3 d in cockroach rearing containers, a water bottle, and 2 dog chow pieces. Thirty male cockroaches were placed in

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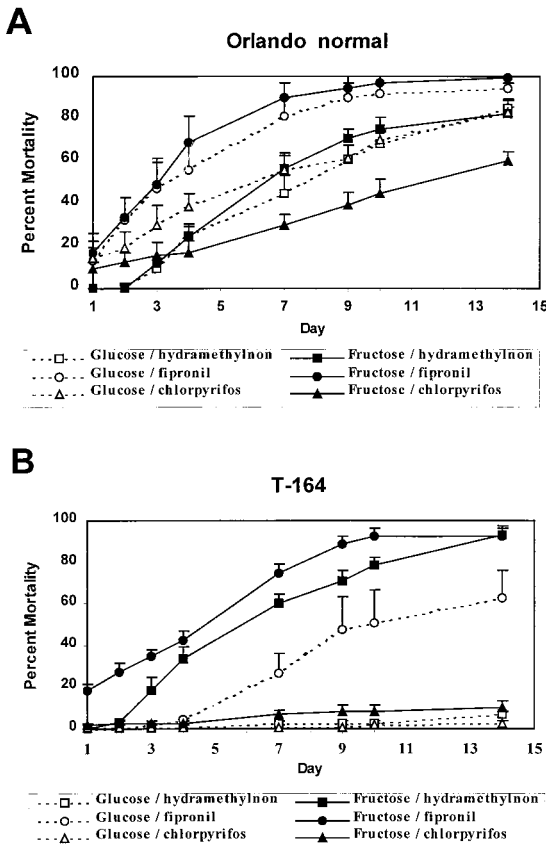


Fig. 1. Percentage mortality (mean ± SE) of (A) Orlando normal and (B) T-164 *B. germanica* exposed to baits containing 3 active ingredients with or without glucose.

each container and allowed to acclimate 3 d before bait placement. Four replicates were conducted for each strain, active ingredient, and corn syrup combination. Mortality was assessed through 14 d, and at each count any dead insects were removed from the containers. The LT_{50} and LT_{90} values were calculated and compared using Weibull distribution analysis (BBN Software Products 1995).

Relative Consumption of Bait. The palatability of each of the insecticides in the 2 bait matrices was determined. Each of the 6 baits (3 active ingredients × 2 corn syrups) was placed in a single container (45 by 30 by 20 cm) housing 300–500 mixed-stage *B. germanica*, which acclimated within the container 7 d before bait placement. Dog chow and water also were provided. Baits were weighed, confined with the cockroaches for 24 h, and then reweighed. Bait consumption data were corrected for water loss (gain) and average consumption per individual cockroach was calculated. Three replicates were conducted for each *B. germanica* strain. The study was repeated by confining only the glucose-containing baits with the T-164 strain to determine whether a difference in bait consumption existed between the glucose baits. Data were analyzed by analysis of variance (ANOVA) with the RSI NWAY procedure and least significance difference (LSD) mean separation test (BBN Software Products 1995).

Toxicity of Insecticides to *B. germanica*. The insecticidal activity of each insecticide against Orlando normal and T-164 cockroaches was determined by topical application. Adult male *B. germanica* were lightly anesthetized with carbon dioxide and a 1 µl droplet of insecticide dissolved in acetone was deposited between the bases of the metacoxae. Twenty cockroaches per strain were treated with each of 5–10 concentrations of fipronil, hydramethylnon, or chlorpyrifos. Mortality was analyzed by probit analysis (BBN Software Products 1995) and the LD_{50} and LD_{90} values (ng per insect) at day 6 were determined.

Results

Nonglucose-averse (Orlando normal) *B. germanica* mortality rates were active ingredient rather than glucose dependent (Fig. 1A; Table 1). However, Orlando normal males exposed to chlorpyrifos and glucose bait died faster than those fed fructose bait.

Fipronil baits without glucose produced slightly faster mortality rates of glucose-averse T-164 cockroaches than did hydramethylnon baits without glucose (Fig. 1B; Table 1). The T-164 males were not affected by the chlorpyrifos bait, even when glucose

Table 1. Mortality of *B. germanica* strains following exposure to various baits

Strain	Insecticide	Glucose (Y/N)	Shape (95% CI)	LT_{50} (CI), d	LT_{90} (CI), d
Orlando normal	Hydramethylnon	Y	2.09 (1.78–2.46)	8.88 (8.02–9.83)	15.78 (14.20–17.53)
		N	1.96 (1.67–2.30)	8.34 (7.48–9.30)	15.38 (13.70–17.27)
	Fipronil	Y	1.34 (1.16–1.55)	4.47 (3.82–5.24)	10.96 (9.46–12.71)
		N	1.48 (1.29–1.70)	3.77 (3.27–4.35)	8.48 (7.47–9.62)
	Chlorpyrifos	Y	1.25 (1.06–1.48)	7.00 (5.90–8.29)	18.25 (15.19–21.92)
		N	1.32 (1.07–1.64)	11.94 (10.03–14.23)	29.63 (22.93–38.31)
T-164	Hydramethylnon	Y	—	—	—
		N	1.95 (1.68–2.26)	7.43 (6.67–8.28)	13.75 (12.41–15.24)
	Fipronil	Y	2.50 (2.05–3.04)	12.02 (10.98–13.17)	19.45 (17.16–22.03)
		N	1.39 (1.19–1.63)	5.13 (4.41–5.97)	12.14 (10.48–14.07)
	Chlorpyrifos	Y	—	—	—
		N	—	—	—

—, Less than 10% mortality. Unable to make estimate.

Table 2. Consumption (mean \pm SD) of insecticidal baits with and without glucose by *B. germanica* 1 d after bait introduction

Strain	Insecticide	Glucose (Y/N)	Population, mg	Individual, mg	% of total
Orlando normal	Hydramethylnon	Y	56.3 \pm 16.74	0.18 \pm 0.059a	11.5
		N	70.0 \pm 29.13	0.21 \pm 0.079a	14.3
	Fipronil	Y	123.3 \pm 2.50	0.38 \pm 0.025a	25.2
		N	111.7 \pm 52.74	0.34 \pm 0.144a	22.9
	Chlorpyrifos	Y	58.7 \pm 7.59	0.18 \pm 0.019a	12
		N	68.7 \pm 30.55	0.21 \pm 0.104a	14.1
T-164	Hydramethylnon	Y	2.7 \pm 3.77	0.01 \pm 0.014c	0.5
		N	194.0 \pm 17.91	0.62 \pm 0.142a	35.7
	Fipronil	Y	8.0 \pm 3.74	0.02 \pm 0.009c	1.5
		N	237.0 \pm 59.20	0.73 \pm 0.113a	43.6
	Chlorpyrifos	Y	0.3 \pm 0.47	0.001 \pm 0.001c	0.06
		N	101.3 \pm 26.39	0.33 \pm 0.147b	18.7

Means for a given time followed by the same letter are not significantly different LSD ($P < 0.05$).

was absent. Hydramethylnon and chlorpyrifos baits with glucose produced little T-164 mortality through 14 d, presumably because little or no bait was consumed. However, the fipronil-glucose bait matrix caused T-164 male mortality beginning on day 7 and reached 60% on day 14.

We compared relative bait consumption and insecticide toxicity to explain fipronil's efficacy against the T-164 strain in the presence of glucose. There was no significant difference ($P = 0.05$) in bait consumption by Orlando normal *B. germanica* given a choice of the 3 active ingredients in a food matrix with or without glucose (Table 2). The T-164 cockroaches given the same 6 bait choices fed overwhelmingly (>97%) on the matrices without glucose, with fipronil and hydramethylnon consumption being similar. Although consumption of the 3 glucose-containing baits was numerically different, extremely low consumption relative to the nonglucose bait made statistical separation impossible. Therefore, T-164 *B. germanica* were subsequently given a choice of each insecticide in a glucose bait only. Glucose baits with fipronil and hydramethylnon were consumed more than chlorpyrifos after 1 d, and fipronil bait consumption was significantly higher than that of the other active ingredients over 4 d (Table 3).

When applied topically, fipronil was 1,150 and 485 times more toxic (LD_{50}) to T-164 males than hydramethylnon and chlorpyrifos, respectively (Table 4). Correcting for the active ingredient levels in each of the baits, we find that fipronil bait was 17 and 29 times more toxic to the T-164 strain than hydramethylnon and chlorpyrifos baits, respectively.

Discussion

The T-164 cockroaches reduced their bait consumption when glucose was present in the formulation, resulting in less ingested toxicant and significantly higher survivorship. The presence of glucose in hydramethylnon bait caused a reduction in bait consumption with a corresponding dramatic reduction in bait efficacy from >90% to <5% mortality, confirming findings by Silverman and Bieman (1993). Only fipronil bait caused significant mortality in German cockroaches exhibiting behavioral resistance to a bait inert. We examined 2 factors that might be responsible for inhibiting the full expression of T-164 behavioral resistance to fipronil baits with glucose: low level bait consumption and high bait toxicity. Although glucose-bait consumption represented <10% of the total bait consumption, more fipronil-glucose bait was consumed than either hydramethylnon-glucose or chlorpyrifos-glucose bait. Average individual T-164 consumption of the 0.03% fipronil-glucose bait in the 6-bait comparison was 0.02 mg (Table 2) provided an ingested dose of 6 ng of fipronil, which is above the topical LD_{90} for fipronil reported herein, and similar to that reported by Scott and Wen (1997) and Valles et al. (1997). When T-164 cockroaches could choose between glucose baits and dog chow, the ingested dose was considerably higher. Also, the bait toxicity of fipronil, calculated from active ingredient concentration and topical LD_{50} , was substantially higher than that of hydramethylnon and chlorpyrifos, which may account for the T-164 mortality in the fipronil-glucose bait experiment. Fipronil is also effective as a contact

Table 3. Consumption (mean \pm SD) of insecticidal baits with glucose by T-164 *B. germanica*

Days after bait introduction	Insecticide	Population, mg	Individual, mg	% of Total
1	Hydramethylnon	49.7 \pm 18.45	0.15 \pm 0.054a	30.9
	Fipronil	106.3 \pm 49.64	0.31 \pm 0.138a	66.1
	Chlorpyrifos	4.7 \pm 6.42	0.01 \pm 0.018b	2.9
4	Hydramethylnon	68.3 \pm 30.00	0.18 \pm 0.052b	26
	Fipronil	183.3 \pm 52.27	0.53 \pm 0.130a	70
	Chlorpyrifos	10.3 \pm 15.37	0.02 \pm 0.044c	4

Means for a given time followed by the same letter are not significantly different LSD ($P < 0.05$).

Table 4. Toxicity of active ingredients to Orlando normal and T-164 male *B. germanica*

Insecticide	Strain	Slope \pm SE	LD ₅₀ (CI) ng/insect	LD ₉₀ (CI) ng/insect	χ^2 (3 df)
Hydramethylnon	Orlando normal	4.62 \pm 0.82	3,978.6 (3,124.3–4,832.8)	6,671.5 (5,029.0–8,314.0)	12.12*
Hydramethylnon	T-164	6.40 \pm 1.32	3,106.6 (2,529.9–3,683.3)	4,498.9 (3,602.9–5,394.9)	0.91
Fipronil	Orlando normal	6.45 \pm 1.38	2.21 (1.84–2.59)	3.06 (2.36–3.76)	0.39
Fipronil	T-164	5.27 \pm 1.00	2.70 (2.27–3.13)	3.96 (3.27–4.65)	0.32
Chlorpyrifos	Orlando normal	8.29 \pm 1.55	338.5 (299.4–377.7)	448.8 (399.2–498.3)	0.04
Chlorpyrifos	T-164	6.12 \pm 1.29	1,309.1 (1,097.3–1,520.9)	1,924.7 (1,574.6–2,274.9)	1.99

*. Not a significant fit ($P < 0.05$).

toxicant (Hamon et al. 1996); consequently, cockroaches might obtain a lethal dose through multiple encounters with the bait surface.

Silverman and Ross (1994) revealed some T-164 mortality with a hydramethylnon–glucose bait matrix similar to that used in the current study. An additional 4 yr of continuous selection with a hydramethylnon bait containing glucose might have amplified the glucose aversion trait, but also may have selected for physiological or behavioral resistance to hydramethylnon. However, there was no evidence for hydramethylnon tolerance in the T-164 strain (Table 4), and a paired comparison of baits with and without hydramethylnon did not reveal a difference in consumption (D.L., unpublished data).

The T-164 *B. germanica* strain was collected from the field in 1990 and has had no exposure to chlorpyrifos at least since then, yet these cockroaches were not killed by the chlorpyrifos baits in this study, even the bait without glucose. Ross (1997) described 2 field strains of *B. germanica* with some physiological resistance to chlorpyrifos that were affected less by a chlorpyrifos bait than a susceptible strain. Behavioral resistance to the chlorpyrifos bait was apparent in these field-derived strains evidenced by bait consumption that decreased through successive generations. Subsequently, Ross (1998) determined that the resistance was caused by aversion to chlorpyrifos. Although behavioral resistance to chlorpyrifos was not measured in the T-164 strain, it (like the persistence of \approx 4-fold physiological resistance) may have been responsible for the poor performance of the chlorpyrifos bait.

Behavioral resistance to inert and active ingredients can compromise the performance of cockroach bait products and may be more common than reported. In instances where insects reject 1 or more bait inerts, minimal ingestion of the bait matrix may render a moderately active toxicant ineffective. Highly active insecticides, such as fipronil, can override these inert-based resistance mechanisms.

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