Behavioral and Physiological Resistance of the German Cockroach to Gel Baits (Blattodea: Blattellidae)

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ABSTRACT A gel bait-resistant German cockroach, Blattella germanica (L.), strain Cincy was collected in Cincinnati, OH. This strain exhibited a high level of behavioral resistance to Avert (0.05% abamectin) and Maxforce FC (0.01% ßpronil) gel baits. Topical application assays indicated moderate levels of physiological resistance of the Cincy strain to abamectin and ßpronil. Resistance ratios (based on LD₅₀ values from topical applications) to abamectin and ßpronil were 2.5 and 8.7, respectively. The Cincy strain had a significantly lower LD₅₀ value to abamectin than a nonaverse field strain (Dorie) and similar LD₅₀ values to ßpronil as the Dorie strain. The aversion behavior (avoidance of gel baits) was therefore caused by food ingredients in the gel baits. The Cincy strain showed avoidance of agar containing fructose, glucose, maltose, and sucrose, which are phagostimulants to the laboratory strain. Modifications of the inert ingredients in the Maxforce FC gel bait significantly improved the efficacy against the Cincy strain. The Cincy strain produced significantly smaller oothecae and lower numbers of eggs in each egg capsule than the nonaverse Jwax and Dorie strains of cockroaches, suggesting fitness costs are associated with resistance.

KEY WORDS Blattella germanica, resistance, food aversion, ßpronil, abamectin

Gel baits have been the main method for German cockroach, Blattella germanica (L.), control in the United States for at least 5–8 yr (Harbison et al. 2003). This popularity was a result of the availability of a variety of highly effective and economical bait products such as Siege (1992), Maxforce (1997), Combat (1997), Avert (1997), Maxforce FC (1998), and PreEmpt (1999). The active ingredients used in these cockroach baits are hydramethylnon, ßpronil, abamectin, and imidacloprid. Gel baits are proven to be convenient to use and highly effective (Appel 1992, Ross 1993, Appel and Benson 1995, Kaakeh et al. 1997, Appel and Tanley 2000). They are also safer and more environmentally friendly than insecticide sprays due to their targeted application. As a result, gel baits have become a very popular cockroach management tool for pest management professionals. When the active ingredient is incorporated into a palatable bait, cockroaches readily consume a lethal dose from a single meal. Therefore, baits were considered less likely to select for high-level cockroach resistance than insecticide sprays and other formulations.

Historical data have shown that B. germanica has the capability to develop resistance to most insecticides in wide-scale use (Cochran 1995, Scharf and Bennett 1995). The majority of resistance research has been on physiological resistance to various neurotoxic active ingredients. Behavioral resistance of B. germanica was reported by Ross (1993, 1998), Silverman and Bieman (1993), and Silverman and Ross (1994). Food aversion, more specifically, glucose aversion previously accounted for the failure of a cockroach bait product in the 1990s (Silverman and Bieman 1993). From 1999, pest management professionals began to report failures of gel baits (Harbison et al. 2003, Morrison et al. 2004). Some field B. germanica populations were found not to feed on gel baits. Logically, this phenomenon was commonly found in locations where poor sanitation conditions and frequent use of gel baits had prevailed. Strong interest developed among researchers, manufacturers, and consumers to understand this emerging behavioral resistance of B. germanica. In 2003, we began to evaluate the behavioral and physiological resistance of B. germanica to cockroach gel baits. We collected a bait-averse strain of B. germanica from multifamily apartments. Four popular gel baits representing four active ingredients (ßpronil, hydramethylnon, abamectin, and imidacloprid) were evaluated. Here, we report results on the behavioral and physiological resistance of two field strains of B. germanica and on fitness and fecundity parameters associated with resistance.

Materials and Methods

Cockroach Strains. Three B. germanica strains were used in this study. Jwax is a standard susceptible strain maintained in the laboratory for >30 yr (Koehler and

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Patterson 1986). It was originally collected before the widespread use of synthetic organic insecticides. The Dorie strain was collected in June and July 2003 from 10 apartments in Gary, IN. These apartments received a variety of pyrethroid sprays, gel baits, bait stations, and boric acid dust by residents, contractors, and researchers from Purdue University each year during the past 5 yr. The Cincy strain was collected in May 2003 from eight apartments in Cincinnati, OH. These apartments were quarterly treated with gel baits for at least 5 yr before collection. This strain showed clear avoidance behaviors toward gel baits based on observations from pest management professionals servicing the apartment building. All of the strains were reared on Harlan Teklad rodent diet (Harlan Teklad, Madison, WI), peanut butter, and mixed fruit jelly (J. M. Smucker Co., Orrville, OH) before experiments. No additional selection pressure was applied to the field strains. Cockroaches were reared in 40.5 by 28.0 by 14.5-cm plastic boxes in walk-in environmental chambers at 26°C, 60% RH, and a photoperiod of 12:12 (L:D) h.

**Efficacy of Gel Baits.** Four cockroach gel bait products were evaluated against the Jwax, Dorie, and Cincy strains. The baits were Maxforce FC Professional Insect Control Roach Killer Bait Gel (0.01% fipronil), Maxforce Professional Insect Control Roach Killer Bait Gel (2.15% hydramethylnon), and Pre-Empt Professional Cockroach Gel Bait (2.15% imidacloprid), manufactured by Bayer Environmental Science (Montvale, NJ), and Avert Cockroach Gel Bait (0.05% Abamectin B1), manufactured by Whitmire Micro-Gen Research Laboratories (St. Louis, MO). Ten adult male cockroaches were counted and placed in each plastic box (18.7 by 13.3 by 9.5 cm) 1 d before bait introduction. The inner upper portion of the boxes was lightly greased with a mixture of petroleum jelly and mineral oil (2:3) to prevent escape. Each box contained a cotton-plugged water vial and a 10 by 10-cm cardboard “tent” as a harborage. Approximately 0.4 g of gel bait in a 0.7-ml centrifuge vial was placed in each cockroach box after the 1-d acclimation period. Each box also received a piece of rodent diet as alternative food. The control box was provided with rodent diet only. Each treatment was applied to three boxes. Cockroach mortality was recorded daily until 7 d. Moribund insects (defined by an inability to walk) were considered dead in these and all other experiments. Experimental units were kept in a walk-in environmental chamber at 26°C, 60% RH, and a photoperiod of 12:12 (L:D) h.

**Topical Assays.** Adult male Jwax, Dorie, and Cincy cockroaches, 1–4 wk old, were removed from rearing containers and deprived of food for 1 d before treatment. Their weights were measured on a Mettler AE100 balance (Mettler-Toledo Inc., Columbus, OH) immediately before treatment. Technical grade fipronil at 96.8% purity (Bayer Environmental Science) or abamectin at 70% purity (Whitmire Micro-Gen Research Laboratories) was applied after dissolving in reagent grade acetone. The insecticide was applied using a Burkard Auto Microapplicator (Burkard Manufacturing Co. Ltd., Hertfordshire, England) equipped with a 1-ml glass syringe. One microliter of insecticide solution was applied onto the first sternite of each CO2-anesthetized cockroach. After application, test insects were placed in groups of 10 in 100 by 25-mm plastic petri dishes with a water vial and cardboard harborage. Each concentration was applied to 30 cockroaches. The concentrations of fipronil were Cincy: 1.2, 3.6, 10.8, 32.4, and 97.2 ppm; Dorie: 0.4, 1.2, 3.6, 10.8, and 32.4 ppm; and Jwax: 0.5, 1, 1.5, 2, and 2.5 ppm. The concentrations of abamectin were Cincy: 2.5, 10, 40, and 50 ppm; Dorie: 2.5, 5, 10, 20, and 40 ppm; and Jwax: 0.25, 1.25, 2.5, 5, 10, and 20 ppm. At least three concentrations causing 1–99% mortality were included for each chemical. The dish lid had a 2.5-cm-diameter screened opening for ventilation. Mortality was observed 72 h after treatment. After treatment, insects were held in a walk-in environmental chamber at 26°C, 60% RH, and a photoperiod of 12:12 (L:D) h.

**Consumption of Blank Gel Baits.** Blank Avert (provided by Whitmire Micro-Gen Research Laboratories) and Maxforce FC (provided by Bayer Environmental Science) gel baits were evaluated against the Jwax, Dorie, and Cincy strains to assess aversion behavior. Fifteen adult males and 15 nongravid adult females were placed in each plastic box 1 d before tests. They were only provided with water. A 0.7-ml centrifuge vial containing blank gel bait and a piece of rodent diet were added to each box on the test date. Three replicate boxes of each cockroach strain were assayed for each bait. The weight of the vials was recorded daily. However, data beyond 2 d were not analyzed because of the onset of low levels of mortality (<10%). A vial containing each of the two baits was placed in a box without cockroaches for estimation of weight loss due to evaporation. Consumption of bait was calculated by the formula \( W_0 - W_n/(1 - \text{water}) \), where \( W_0 \) and \( W_n \) are the weight before and after exposure to cockroaches, respectively, and \( \text{water} \) is the percentage of water loss in the control vial. Experiment boxes were kept in a walk-in environmental chamber at 26°C, 60% RH, and a photoperiod of 12:12 (L:D) h.

**Sugar Consumption Assay.** This experiment was conducted to identify food elements that might contribute to the bait aversion. Sugar-agar diets of each of the sugars D-fructose, D-galactose, D-glucose, D-maltose, and D-sucrose were made by adding sugar [15% (wt:wt)] and agar [1% (wt:wt)] in deionized water, bringing to a boil, and then pouring into 100 by 25-mm sterile petri dishes. The diet was stored at 4°C before use. Mixed age populations of the Jwax, Dorie, and Cincy strains were tested. They were maintained in 40.5 by 28.0 by 14.5-cm plastic boxes. Each box contained 200-1000 individuals. Food was removed 48 h before feeding trials to facilitate a rapid response to the diets. A cube of each diet was placed in a weigh dish and weighed. Seven diets (six sugar-agar diets and one agar diet) were placed in each cockroach rearing box. Four to six replicate boxes of each strain were assayed. The diets were again
weighed after 24 h of exposure. A set of diets was placed in a box without cockroaches for estimation of water loss. Consumption of diets was calculated by the same method used in the blank bait consumption experiment. The feeding index was calculated as \((W_s - W_a)/T\), where \(W_s\) and \(W_a\) are consumption of sugar-agar diet and agar diet, respectively, and \(T\) is the total consumption of the seven diets in each box. A positive number indicates that a sugar stimulates feeding. A negative number indicates the sugar deters feeding. Experimental units were kept in a walk-in environment chamber at 26°C, 60% RH, and a photoperiod of 12:12 (L:D) h.

**Comparative Efficacy of Original and New Maxforce Gel Bait Formulations.** Comparative efficacy of original (Maxforce FC) and new Maxforce gel bait (Maxforce FC Select) formulations containing 0.01% fipronil or 2.15% hydramethylnon were evaluated against the Cincy strain. These baits were provided by Bayer Environmental Science. The original formulations refer to the currently marketed formulations, i.e., Maxforce FC Professional Insect Control Roach Bait (0.01% fipronil) and Maxforce Professional Insect Control Roach Killer Bait Gel (2.15% hydramethylnon). The new formulation was designed to overcome the aversion behavior in the \(B.\) germanica populations. Different sugar and other food ingredients were used in the new formulation (G. Braness, personal communication). Ten adult male cockroaches (2–3 wk old) were placed into each plastic box (18.7 by 13.3 by 9.5 cm). Test roaches were provided with a block of rodent chow and 0.5 g of gel bait that was placed in a 0.7-ml centrifuge vial. Each treatment was provided to three replicate boxes of cockroaches. The control boxes only received rodent chow as food. Mortality was recorded daily for 11 d. Dead cockroaches were removed during each observation. Experiment boxes were kept at 21°C, 31% RH, and approximately a photoperiod of 12:12 (L:D) h.

**Ootheca Measurement.** After the first cycle of oothecae produced by gravid females, the empty oothecae were collected and measured under an Olympus SZ-60 (Olympus Optical Co., Tokyo, Japan) dissecting scope at 10× magnification. The length and number of embryo chambers (defined by transverse lines) of the oothecae were recorded.

**Data Analysis.** Ootheca length and number of embryo chambers, mortality (arcsine of the square-root transformed), blank bait consumption, and feeding index data were analyzed by analysis of variance (ANOVA) by using SAS software (SAS Institute 2000). Means of the blank bait consumption, feeding index of agar-sugar diet, and ootheca measurements of the three strains were separated by Tukey’s test. Means of the bait efficacy among the three strains were separated by Dunnett’s test. The pooled number of dead cockroaches in each concentration from topical assay was analyzed by Proc Probit in SAS software. LD\(_{50}\) and LD\(_{90}\) values were estimated for each strain and each tested insecticide, with corresponding resistance ratios.

**Results**

**Efficacy of Gel Baits.** After 7-d exposures, all baits caused high mortality to the Jwax and Dorie strains (Table 1). The Cincy strain exhibited high levels of resistance to Avert, Pre-Empt, and Maxforce FC gel baits compared with Jwax. The Cincy strain was more resistant to Avert, Pre-Empt, and Maxforce FC baits than the Dorie strain. Only Maxforce (2.15% hydramethylnon) caused significant mortality to the Cincy strain \((F = 9.1; \ df = 4, 10; P = 0.002)\). The laboratory Jwax strain exhibited much higher mortality at 4 d postexposure to Avert, Maxforce, and Pre-Empt than the two field strains.

**Topical Assays.** LD\(_{50}\) and LD\(_{90}\) values indicate that moderate \((i.e., <10\text{-fold at } \text{LD}_{50})\) levels of physiological resistance to abamectin and fipronil are present in the Cincy and Dorie strains (Table 2). The Cincy strain has lower levels of resistance to abamectin than the Dorie strain. However, in the gel bait efficacy experiment, the Cincy strain exhibited higher levels of resistance to Avert bait than the Dorie strain. This demonstrates that behavior likely plays the major role in the lack of efficacy on the Cincy strain by Avert gel bait. Similarly, the Cincy strain seems to also have

<table>
<thead>
<tr>
<th>Gel bait</th>
<th>Cockroach strain</th>
<th>n</th>
<th>4d</th>
<th>7d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avert (0.05% Abamectin B1)</td>
<td>Jwax</td>
<td>3</td>
<td>93.3 ± 3.3a</td>
<td>100.0 ± 0.0a</td>
</tr>
<tr>
<td></td>
<td>Dorie</td>
<td>3</td>
<td>69.3 ± 5.1b</td>
<td>96.7 ± 3.3a</td>
</tr>
<tr>
<td></td>
<td>Cincy</td>
<td>3</td>
<td>0.0 ± 0.0c</td>
<td>0.0 ± 0.0b</td>
</tr>
<tr>
<td>Maxforce FC (0.01% fipronil)</td>
<td>Jwax</td>
<td>3</td>
<td>100.0 ± 0.0a</td>
<td>100.0 ± 0.0a</td>
</tr>
<tr>
<td></td>
<td>Dorie</td>
<td>3</td>
<td>100.0 ± 0.0a</td>
<td>100.0 ± 0.0a</td>
</tr>
<tr>
<td></td>
<td>Cincy</td>
<td>3</td>
<td>16.7 ± 1.2b</td>
<td>37.5 ± 13.8b</td>
</tr>
<tr>
<td>Maxforce (2.15% hydramethylnon)</td>
<td>Jwax</td>
<td>3</td>
<td>100.0 ± 0.0a</td>
<td>100.0 ± 0.0a</td>
</tr>
<tr>
<td></td>
<td>Dorie</td>
<td>3</td>
<td>10.0 ± 5.8b</td>
<td>93.0 ± 3.5b</td>
</tr>
<tr>
<td></td>
<td>Cincy</td>
<td>3</td>
<td>33.3 ± 8.8c</td>
<td>85.0 ± 5.0b</td>
</tr>
<tr>
<td>Pre-Empt (2.15% hydramethylnon)</td>
<td>Jwax</td>
<td>3</td>
<td>90.0 ± 0.0a</td>
<td>100.0 ± 0.0a</td>
</tr>
<tr>
<td></td>
<td>Dorie</td>
<td>3</td>
<td>70.0 ± 6.5b</td>
<td>80.9 ± 9.5b</td>
</tr>
<tr>
<td></td>
<td>Cincy</td>
<td>3</td>
<td>3.3 ± 3.3c</td>
<td>13.3 ± 8.8c</td>
</tr>
</tbody>
</table>

* Mortality was corrected by the formula in Abbott (1925). Different letters indicate significant differences between strains at \(\alpha = 0.05\).
developed high levels of behavioral, rather than physiological resistance to Maxforce FC gel bait.

Consumption of Blank Gel Baits. After blank Avert gel baits were placed into assay boxes, Jwax and Dorie strain cockroaches were seen feeding on the gel bait within 0.5 h. There was no detectable feeding by the Cincy strain after 2 d. Mean consumption (2 d) of blank Avert bait by the Jwax, Dorie, and Cincy strains were 0.29 ± 0.01, 0.19 ± 0.01, and 0.00 ± 0.00 g, respectively. The relative consumption levels were Jwax > Dorie > Cincy (F = 360.7; df = 2, 6; P < 0.001). Mean consumption (2 d) of blank Maxforce FC gel bait by the Jwax, Dorie, and Cincy strains were 0.29 ± 0.01, 0.19 ± 0.01, and 0.00 ± 0.00 g, respectively. The relative consumption levels were Jwax > Dorie > Cincy (F = 28.9; df = 2, 6; P = 0.001). This experiment verifies the existence of aversion (i.e., behavioral resistance) by the Cincy strain to Avert and Maxforce FC baits. The aversion is apparently caused by inert ingredients in the baits.

Sugar Preference Assay. Four of the tested sugars stimulated the feeding response of Jwax strain cockroaches (Fig. 1). They were maltose, sucrose, glucose, and fructose. In contrast, all of these four sugars deterred the feeding response of the Cincy strain. The feeding response of the Dorie strain was intermediate between Jwax and Cincy. Only maltose and sucrose stimulated feeding of the Dorie strain. Relative feeding levels of the four sugars (maltose, sucrose, glucose, and fructose) were Jwax > Dorie > Cincy. Relative activity of the four sugars was maltose > sucrose > glucose > fructose. (F = 42.9; df = 11, 52; P < 0.001).

Comparative Efficacy of New and Original Maxforce Gel Bait Formulations. The new gel matrix containing either fipronil or hydramethylnon has significantly improved efficacy against the Cincy strain compared with the original formulations (Fig. 2). The original Maxforce FC (0.01% fipronil) caused only 20% mortality after 7-d exposure. In contrast, the new formulation with 0.01% fipronil caused 100% mortality after 4-d exposure. Similarly, new hydramethylnon gel bait caused 100% mortality at 11 d, whereas the original formulation caused only 36.7 ± 8.8% mortality at 11 d.

Ootheca Size. There were clear differences in the size of the oothecae produced by the three strains of B. germanica (Table 3). The average length of the oothecae was Jwax > Dorie > Cincy (F = 127.6; df = 2, 142; P < 0.001). The number of embryo chambers

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**Table 2. Susceptibility of the three strains of B. germanica to two topically applied insecticides**

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Cockroach strain</th>
<th>n*</th>
<th>Model parametersb</th>
<th>Lethal dose (µg/g)c</th>
<th>RR50 d</th>
<th>RR90 e</th>
<th>Model fit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intercept ± SE</td>
<td>Slope ± SE</td>
<td>LD50 (95% FL)</td>
<td>LD90 (95% FL)</td>
<td>d</td>
<td>e</td>
<td>χ², df, P</td>
</tr>
<tr>
<td>Abamectin</td>
<td>Jwax 210</td>
<td>-2.07 ± 0.50</td>
<td>3.67 ± 0.70</td>
<td>0.08 (0.06-0.10)</td>
<td>0.18 (0.13-0.25)</td>
<td>1.18</td>
<td>4 0.88</td>
</tr>
<tr>
<td></td>
<td>Cincy 150</td>
<td>-2.26 ± 0.48</td>
<td>2.31 ± 0.36</td>
<td>0.20 (0.12-0.30)</td>
<td>0.72 (0.49-1.26)</td>
<td>2.5</td>
<td>3 0.35</td>
</tr>
<tr>
<td></td>
<td>Dorie 180</td>
<td>-3.14 ± 0.50</td>
<td>2.26 ± 0.39</td>
<td>0.54 (0.39-0.85)</td>
<td>2.01 (1.18-5.34)</td>
<td>6.8</td>
<td>10 0.36</td>
</tr>
<tr>
<td>Fipronil</td>
<td>Jwax 180</td>
<td>-1.24 ± 0.35</td>
<td>8.23 ± 1.47</td>
<td>0.030 (0.026-0.033)</td>
<td>0.043 (0.039-0.050)</td>
<td>4.02</td>
<td>3 0.26</td>
</tr>
<tr>
<td></td>
<td>Cincy 150</td>
<td>-1.57 ± 0.40</td>
<td>1.39 ± 0.31</td>
<td>0.26 (0.06-1.00)</td>
<td>2.13 (0.66-4.46)</td>
<td>8.7</td>
<td>44.9 0.08</td>
</tr>
<tr>
<td></td>
<td>Dorie 180</td>
<td>-1.59 ± 0.26</td>
<td>1.41 ± 0.24</td>
<td>0.28 (0.17-0.35)</td>
<td>2.25 (1.09-9.84)</td>
<td>9.3</td>
<td>52.7 0.20</td>
</tr>
</tbody>
</table>

a Total number of insects treated with 30 insects in each concentration.
b The intercept and slope parameters are for models in which the independent variable is natural logarithm of dose (ppm).
c Dose (micrograms of insecticide per gram of insect) calculated based on body weights. Average body weights (mean ± SEM) per strain (n = 30) were Jwax, 0.048 ± 0.007 g; Cincy, 0.051 ± 0.001 g; and Dorie, 0.047 ± 0.004 g.
d Resistance ratio based on LD50 values compared with Jwax.

e Resistance ratio based on LD90 values compared with Jwax.

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**Fig. 1. Feeding index of sugar-agar diets (mean ± SEM) by B. germanica.** F, d-fructose; G, d-galactose; GLU, d-glucose; L, d-lactose; M, d-maltose; S, d-sucrose.

**Fig. 2. Comparative efficacy (mean ± SEM) of original and new Maxforce gel baits against the Cincy strain of B. germanica.** Mean of three replicates with 10 adult males in each replicate. Fip-Old, Maxforce FC (0.01% fipronil); Fip-New, Maxforce FC new formulation (0.01% fipronil); Hyd-Old, Maxforce FC new formulation (0.15% hydramethylnon); Hyd-New, Maxforce new formulation (2.15% hydramethylnon).
The avoidance of these common sugars such as sucrose, fructose, glucose, and maltose (Food Standards Agency 2002) have sugars such as fructose. Avert gel bait contains glucose, fructose, and maltose. Maxforce FC gel bait contains sucrose, fructose, and maltose (Food Standards Agency 2002). The emergence of bait aversion poses new challenges to cockroach management professionals. There are two lessons we may learn from this phenomenon. First, rotating gel baits containing different active ingredients apparently will not circumvent this form of resistance. Second, cockroaches will likely develop similar behavioral resistance in response to other inert gel bait matrix ingredients after repeated exposure. The ability of cockroaches to develop behavioral resistance reiterates the importance of using integrated pest management for long-term management of cockroaches. Modifications to gel bait formulations can improve the feeding response in field B. germanica populations. Our study verifies that new gel bait formulations with 0.01% fipronil or 2.15% hydramethylnon are palatable to cockroaches which showed aversion behaviors to the original gel bait formulations. Although better control can be achieved by changing the bait matrix, efficacy is likely to diminish over time if current management practices are not revised.

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