Multiple Mechanisms Confer Fipronil Resistance in the German Cockroach: Enhanced Detoxification and \textit{Rdl} Mutation

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Abstract

Populations of \textit{Blattella germanica} (L.) (German cockroach) have been documented worldwide to be resistant to a wide variety of insecticides with multiple modes of action. The phenylpyrazole insecticide fipronil has been used extensively to control German cockroach populations, exclusively in baits, yet the highest reported fipronil resistance is 38-fold in a single population. We evaluated five populations of German cockroaches, collected in 2018–2019 in apartments in North Carolina and assayed in 2019, to determine the status of fipronil resistance in the state. Resistance ratios in field-collected strains ranged from 22.4 to 37.2, indicating little change in fipronil resistance over the past 20 yr. In contrast, resistance to pyrethroids continues to escalate. We also assessed the roles of detoxification enzymes in fipronil resistance with four synergists previously shown to diminish metabolic resistance to various insecticides in German cockroaches—piperonyl butoxide, S,S,S-tributyl phosphorotrithioate, diethyl maleate, and triphenyl phosphate. These enzymes appear to play a variable role in fipronil resistance. We also sequenced a fragment of the \textit{Rdl} (\textit{resistant to dieldrin}) gene that encodes a subunit of the GABA receptor. Our findings showed that all field-collected strains are homozygous for a mutation that substitutes serine for an alanine (A302S) in \textit{Rdl}, and confers low resistance to fipronil. Understanding why cockroaches rapidly evolve high levels of resistance to some insecticides and not others, despite intensive selection pressure, will contribute to more efficacious pest management.

Key words: urban IPM, German cockroach, \textit{Blattella germanica}, fipronil, insecticide resistance, \textit{Rdl}, target site insensitivity

The German cockroach (\textit{Blattella germanica} L., Blattodea: Ectobiidae) is arguably the most prevalent and harmful indoor pest in the United States, and often the most difficult to eradicate, especially from multi-family apartment complexes. Chronic cockroach infestations produce potent aeroallergens that trigger allergies and asthma in atopic children (Rosenstreich et al. 1997, Pomés and Schal 2020). Cockroaches also harbor a diverse microbial community in their digestive system and feces (Kakumanu et al. 2018) and have been implicated in pathogen transmission in hospitals and residential settings (Schal and DeVries 2021). Controlling and eradicating cockroach infestations is the most effective strategy to mitigate potential health risks (Schal and Hamilton 1990, Schal and DeVries 2021). However, German cockroach eradication strategies are seriously constrained by the rapid evolution of resistance to numerous active ingredients across a wide array of modes of action (Scharf and Gondhalekar 2021).

Strategies to manage German cockroach populations in residential settings have transitioned from residual insecticides to various bait formulations (Appel and Rust 2021). Baits offer several noteworthy advantages over sprays. Namely, they target the pest more effectively, the active ingredient in baits is more bioavailable, and baits leave significantly less residues on household surfaces (DeVries et al. 2019a). Moreover, there is a much wider assortment of active ingredients available for use in bait formulations than...
in sprays, and the dose that insects receive from ingesting baits is typically higher than in sprays, representing a more effective ‘high dose’ strategy.

Fipronil has been used in baits for cockroach control for nearly three decades (Kaakeh et al. 1997). It is a member of the broad-spectrum phenylpyrazole class of insecticides that act as antagonists of gamma-aminobutyric acid (GABA)-gated chloride channels (Gant et al. 1998). Fipronil blocks both GABA-gated channels that mediate synaptic inhibition in the insect central nervous system (Gant et al. 1998) and glutamate-gated chloride (GluCl) channels involved in locomotion, feeding and sensory input (Zhao et al. 2004, Narahashi et al. 2010). It was first registered in the United States for cockroach control in 1996, but 1 yr later, resistance was reported in the German cockroach (Scott et al. 1997, Valles et al. 1997). Both reports documented low levels of resistance ranging from a resistance ratio based on the LD_{50} value (RR) of 1.3 in three cockroach strains (Valles et al. 1997) to 7 in seven strains (Scott et al. 1997) (Table 1). Holbrook et al. (2003) collected cockroaches from 20 populations in central North Carolina before fipronil baits were introduced in commercial products and found moderate and highly variable levels of resistance, ranging from 1.2 to >17-fold (Table 1). However, despite intensive selection pressure with baits over the past three decades, resistance levels have increased only marginally, to 17–38-fold (Wang et al. 2004, Gondhalekar et al. 2012, Lee et al. 2022a).

Table 1. Summary of studies that quantified fipronil resistance in field-collected *B. germanica*

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>RR * range</th>
<th>No. of strains</th>
<th>Collection years</th>
<th>Location</th>
<th>Type of treatment</th>
<th>Assay used</th>
<th>Time of mortality assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>Scott and Wen</td>
<td>1.0–1.8, (1.8–7.7) *</td>
<td>7</td>
<td>1990–1992</td>
<td>United States</td>
<td>Topical</td>
<td>Dose-response</td>
<td>4 d</td>
</tr>
<tr>
<td>1997</td>
<td>Valles et al.</td>
<td>1.0–1.3</td>
<td>3</td>
<td>1989–1996</td>
<td>United States</td>
<td>Topical</td>
<td>Dose-response</td>
<td>1 d</td>
</tr>
<tr>
<td>2004</td>
<td>Wang et al. *</td>
<td>8.7–9.3</td>
<td>3</td>
<td>2003</td>
<td>United States</td>
<td>Topical</td>
<td>Dose-response</td>
<td>3 d</td>
</tr>
<tr>
<td>2006</td>
<td>Nasrian et al.</td>
<td>1–2.6</td>
<td>11</td>
<td>Unknown</td>
<td>Iran</td>
<td>Topical</td>
<td>Dose-response</td>
<td>3 d</td>
</tr>
<tr>
<td>2010</td>
<td>Chai and Lee</td>
<td>1.0–10.0</td>
<td>22</td>
<td>2005</td>
<td>Singapore</td>
<td>Topical</td>
<td>Dose-response</td>
<td>2 d</td>
</tr>
<tr>
<td>2012</td>
<td>Gondhalekar et al.</td>
<td>3.7</td>
<td>1</td>
<td>2006</td>
<td>United States</td>
<td>Topical</td>
<td>Dose-response</td>
<td>3 d</td>
</tr>
<tr>
<td>2013</td>
<td>Ang et al.</td>
<td>1.2–3.0 (10.8–25.8) *</td>
<td>6 *</td>
<td>2005</td>
<td>Singapore</td>
<td>Topical</td>
<td>Dose-response</td>
<td>2 d</td>
</tr>
<tr>
<td>2016</td>
<td>Ko et al.</td>
<td>5.6 (15.9) *</td>
<td>1</td>
<td>2012</td>
<td>Puerto Rico</td>
<td>Topical</td>
<td>Dose-response</td>
<td>2 d</td>
</tr>
<tr>
<td>2017</td>
<td>Liang et al.</td>
<td>0.9–1.4 (2.5–25.0) *</td>
<td>3</td>
<td>1999–2004</td>
<td>United States</td>
<td>Topical</td>
<td>Dose-response</td>
<td>5 d</td>
</tr>
<tr>
<td>2017</td>
<td>Wu and Appel</td>
<td>2.0–8.7</td>
<td>6</td>
<td>2011–2012</td>
<td>United States</td>
<td>Topical</td>
<td>Dose-response</td>
<td>3 d</td>
</tr>
<tr>
<td>2019b</td>
<td>DeVries et al.</td>
<td>6–23</td>
<td>7</td>
<td>2011–2014</td>
<td>United States</td>
<td>Topical</td>
<td>Dose-response</td>
<td>2 d</td>
</tr>
<tr>
<td>2020</td>
<td>Hu et al.</td>
<td>1.5–3.8</td>
<td>24</td>
<td>2017–2018</td>
<td>Taiwan</td>
<td>Surface contact</td>
<td>Time-course (LT_{50})</td>
<td>7 d</td>
</tr>
<tr>
<td>2022a</td>
<td>Lee et al.</td>
<td>~27.7 *</td>
<td>5</td>
<td>2018–2020</td>
<td>United States</td>
<td>Ingestion</td>
<td>Discriminating doses</td>
<td>3 d</td>
</tr>
<tr>
<td>2022</td>
<td>Present paper</td>
<td>22.4–37.2</td>
<td>5</td>
<td>2018–2019</td>
<td>United States</td>
<td>Topical</td>
<td>Dose-response</td>
<td>4 d</td>
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</table>

RR is the resistance ratio, calculated as LD_{50} (or LT_{50}) of field-collected strain/ LD_{50} (or LT_{50}) of a reference susceptible strain. 
Artificially selected population(s) 
RR >17 is based on the observation that the LD_{50} of the susceptible strain was 2 ng, and 34.5 ng (10-fold the LD_{50}) failed to kill 50% of the cockroaches. 
One of the strains (Cincy) also was used by Wang et al. (2004) and had an RR = 8.6. 
The same 6 populations were examined by Chai and Lee (2010) and Ang and Lee (2011). 
RR >27.7 is based on the observation that the LD_{50} of the susceptible strain was 1.3 ng, and 36 ng (10-fold the LD_{50}) killed 20–70% of the cockroaches.
A mutation that results in substitution of serine or glycine in place of an alanine residue (A302S/G) in RdL confers different levels of resistance in various insect species, and the response to various phenylpyrazole insecticides varies across species and populations of the same species (Zhao et al. 2003, Nakao 2017). In B. germanica, the RdL mutation appears to contribute more to dieldrin resistance than to fipronil resistance (Scott et al. 1997, Hansen et al. 2005, Kristensen et al. 2005, Gondhalekar and Scharf 2012, Ang et al. 2013, Lee et al. 2022b).

High levels of insecticide resistance can severely undermine interventions to eradicate German cockroach infestations. Resistance to most insecticides, such as pyrethroids, evolves rapidly, often within <5 yr after their extensive use against B. germanica (Fardsi et al. 2019, Tang et al. 2019). Despite extensive and intensive selection pressure from fipronil-containing baits, the highest recorded resistance level is approximately 38-fold, in a single strain (Gondhalekar and Scharf 2012, Ang et al. 2013). 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of the Orlando Normal strain, as described for fipronil, to generate a dose-response curve and estimate the LD<sub>50</sub> from Probit analysis. The field-collected cockroaches were then treated with the estimated LD<sub>50</sub> of the Orlando Normal strain, as well as 10-fold and 100-fold the LD<sub>50</sub>. Mortality was assessed 48 h post-treatment. We also weighed individual males of these strains, as well as the Orlando Normal strain.

Detection of Rdl Mutation
Ten adult males from each of the six cockroach populations were screened for the presence of the Rdl mutation that results in the A302S substitution. The head of individual cockroaches was homogenized for 30 s with glass beads in a FastPrep 24 5G homogenizer (MP Biomedicals, Solon, OH) and genomic DNA was extracted using the DNeasy Blood & Tissue extraction kit (Qiagen, Germantown, MD). ATL solution (180 µl) and 20 µl of proteinase-K were added to the homogenized samples and incubated at 56°C for 4 h. The rest of the protocol followed the manufacturer’s instructions. DNA was eluted in 50 µl sterile nuclease-free water and stored at −20°C until further use.

A 245-bp fragment of the GABA receptor gene that includes the Rdl mutation site was amplified with the primers BG-Rdl-F (5ʹ-GTCCGCTTCATGGGATACTA-3ʹ) and BG-Rdl-R (5ʹ-AACGACCCGAAGACCATAC-3ʹ) designed by Hansen et al. (2005). The reactions were conducted in 20 µl reaction mix comprising 10 µl of AmpliTaq Gold 360 2X Master mix (Applied Biosystems, Waltham, MA), 1 µl of 10 µM of each primer, 0.2 µl BSA (bovine serum albumin; Sigma–Aldrich) (20 mg/ml), and 2 µl of German cockroach genomic DNA as template for the PCR reaction. A negative control with no template DNA was included in every PCR run. The following thermal cycle program was used for amplification: Initial activation at 95°C for 10 min followed by 40 cycles of 94°C for 30 s, 64.3°C for 30 s, and 72°C for 30 s and a final extension at 72°C for 10 min (Gondhalekar and Scharf 2012). Two microliters of each PCR product were used to verify proper sized bands on 1.2% agarose gel. The remaining PCR product was purified by ExoSAP-IT (Applied Biosystems) and direct-sequenced at the Genomic Sequencing Laboratory (North Carolina State University, Raleigh, NC) with BG-Rdl-R as sequencing primer. Each sequence was determined by manually checking for the GCC to TCC mutation site.

Table 3: The LD<sub>50</sub> values were determined from dose-response curves 4 d after topical applications of fipronil to cockroaches collected from five apartments (VS101, DR2800, DR2820B, CC29, PR515F; Fig. 1); these values were compared with the insecticide-susceptible Orlando Normal strain. The Orlando Normal males had 97.5% mortality at the highest dose of 3 ng fipronil per insect, whereas 90 ng/insect killed 73–93% of the field-collected cockroaches. The LD<sub>50</sub> of the Orlando Normal males was 1.55 ng/insect, whereas PR513F, the strain with the highest fipronil resistance, had an LD<sub>50</sub> of 57.6 ng/male (Table 2). The resistance ratio values based on their respective LD<sub>50</sub> values ranged from 22.4 (DR2800) to 37.2 (PR515F), and all five strains significantly differed from the Orlando Normal reference strain (P < 0.05). The slopes of the dose–response curves (range, 1.36–3.92) for the five populations were less steep than for the Orlando Normal population (slope = 5.40), and indeed, the RR values at LD<sub>50</sub> were 1.23–3.83 greater than at LD<sub>90</sub> indicating a more heterogeneous response of the field-collected cockroaches. Male body mass values were available for four strains. Their body masses were 0.8%–10.9% greater than the Orlando Normal cockroaches.

We found relatively high levels of cypermethrin resistance in the three populations tested (Table 3). The LD<sub>50</sub> of the Orlando Normal strain was 0.112 µg/male. At a diagnostic dose of 10 µg/male, representing ~100-fold the Orlando Normal LD<sub>50</sub>, only 40% of adult males of strain DR2820B died, indicating a resistance ratio >100. At the same dose of 10 µg/male we found 80% and 83% mortality in strains CC29 and VS101, respectively. These results indicate resistance ratios to cypermethrin between 10 and >100.

Effects of Enzyme Inhibitors on Fipronil Toxicity
The toxicity of fipronil in the presence of the enzyme inhibitors PBO, DEF, DEM, and TPP 4 d after treatment is shown in Fig. 2. Because the fipronil LD<sub>50</sub> varied among the six strains, we used the strain-specific LD<sub>50</sub> dose with and without the addition of the inhibitor. When pretreated with PBO, neither the susceptible reference Orlando Normal strain nor PR515F, the most resistant strain, experienced a significant increase in fipronil toxicity (P = 0.12 and 0.06, respectively). However, fipronil mortality increased significantly in the presence of PBO in strain VS101 (χ<sup>2</sup> = 11.4, df = 1, P < 0.05) and in strain DR2800 (χ<sup>2</sup> = 10.7, df = 1, P < 0.05). Fipronil toxicity also was significantly enhanced in strains DR2820B (χ<sup>2</sup> = 4.6 df = 1, P < 0.05) and CC29 (χ<sup>2</sup> = 6.9 df = 1, P < 0.05), but to a lesser extent than in VS101 and DR2800.

Pre-applications of DEF, an EST inhibitor, caused a significant increase in fipronil mortality only in cockroaches from the VS101 strain (χ<sup>2</sup> = 4.59 df = 1, P < 0.05). DEM, a GST inhibitor, caused significant synergism only in the Orlando Normal susceptible strain and the VS101 strain. In contrast, pre-treatments with TPP, also an EST inhibitor, antagonized the toxicity of fipronil in the susceptible strain, reducing fipronil mortality from 37% to 0%. Our findings suggest an involvement of P450s in fipronil metabolism in most strains, but the activity of the other enzyme inhibitors varied among the strains, with significant antagonism observed with TPP in the susceptible strain (Fig. 2).

### A302S Rdl Mutation
A 245 bp fragment of the GABA receptor gene targeting the position of the Rdl mutation that causes the A302S substitution was
amplified and sequenced from the Orlando Normal susceptible strain \((n = 10)\) and the five field-collected populations \((n = 10\) per population). Based on previous studies relating fipronil resistance to this mutation, and the moderate level of resistance we detected in some populations, we expected to detect haplotypes corresponding to homozygous susceptible wild-type \((\text{Ala}302/\text{Ala}302; S/S)\), putatively homozygous resistant \((\text{Ser}302/\text{Ser}302; R/R)\) and the heterozygous haplotype \((\text{Ala}302/\text{Ser}302; S/R)\). However, we found the \text{Rdl} mutation in all five recently collected cockroach strains, but not in the reference insecticide susceptible strain (Table 4); 100% of the apartment-collected cockroaches were homozygous for the resistant genotype \((A302S)\) (Fig. 3).

### Discussion

It appears that fipronil resistance in German cockroach populations in North Carolina has increased over the last two decades from a range of 1.2–17-fold (Holbrook et al. 2003) to 22.4–37.2-fold. The fipronil LD_{50} values ranged from 34.6 ng/male (approximately 0.69 µg/g) to 57.6 ng/male (approximately 1.15 µg/g). The moderate increase in resistance, despite the prevalence of fipronil-containing baits in the United States, is consistent with patterns in other United States and global populations investigated over the last two decades (Table 1). These findings suggest that fipronil resistance in German cockroach populations might have reached a plateau, possibly representing a level above which
cockroaches experience substantial fitness costs. However, unlike the broad variation in resistance ratios documented in populations in the 1990s and 2000s, we observed low variation among the five German cockroach populations collected in homes in Raleigh, NC. These results support the idea that populations might have reached an upper limit of resistance, represented by RR values of 30–40.

Mechanisms of Fipronil Resistance

The involvement of metabolic enzymes in fipronil degradation appears to vary among cockroach strains. Piperonyl butoxide was reported by Valles et al. (1997) to have antagonistic effects on fipronil toxicity in three different populations, and by Ang et al. (2013) in six populations. On the other hand, Gondhalekar et al. (2012) showed that PBO synergized fipronil in one strain and Lee et al. 2022b showed similar results in four out of five strains, as in our study. However, fipronil mortality in our most resistant strain (PR515F) increased by only 24% in the presence of PBO (at the strain-specific LD₅₀ level of fipronil), from 53% to 77%, and this increase was not statistically significant. Responses to PBO vary among insect species, with synergism in Musca domestica L. (Diptera: Muscidae) (Liu and Yue 2000) and bed bug (González-Morales et al. 2021), but no apparent effect in Diabrotica virgifera virgifera LeConte (Coleoptera: Chrysomelidae) (Scharf et al. 2000). It is important to note that fipronil would be oxidized by P450s to fipronil sulfone, which also binds strongly to GABA and GluCl receptors in insects, including the German cockroach (Zhao and Salgado 2010), and thus plays an important role in the toxicity of fipronil. In vivo studies have found that fipronil and its sulfone metabolite range from similar bioactivity in some insects (e.g., D. virgifera; Scharf and Siegfried 1999), to the sulfone being 4.6-fold more bioactive than fipronil (non-biting midge, Chironomus dilutus Shobanov, Kiknadze & Butler, Diptera; Weston and Lydy 2014). It is possible that populations of German cockroaches express polymorphisms in the affinities of fipronil and fipronil sulfone to GABA and GluCl receptors. Thus, in some cockroach populations the application of PBO blocks fipronil oxidation, but does not greatly affect toxicity because fipronil and the sulfone are equally bioactive. In populations where the sulfone is more bioactive than fipronil, PBO would block the oxidation and thus antagonize fipronil. In yet other populations, P450s may further hydroxylate the sulfone to less active forms. In these populations, PBO might block these later transformations and both fipronil and the sulfone would be more toxic in the presence of PBO. However, the high synergism expected in the latter scenario has not been demonstrated in any German cockroach population.

Similarly, DEF, an EST inhibitor, has been reported to antagonize fipronil toxicity in multiple populations of the German cockroach (Valles et al. 1997, Ang et al. 2013), but synergize fipronil...
in the one investigated strain (Gondhalekar et al. 2012) and four out of five strains (Lee et al. 2022b). Although all five of our field-collected strains had greater mortality after DEF treatment, only one (VS101) showed a significant increase in mortality after DEF application. There was no apparent effect of DEM, a GST inhibitor, on fipronil metabolism in five strains of the German cockroach (Lee et al. 2022b). Interestingly, DEM significantly synergized fipronil toxicity only in the susceptible strain (Orlando Normal) and the VS101 strain, and it slightly elevated mortality in two strains and depressed fipronil mortality in two other strains. The only significant antagonism in our assays was caused by TPP in the Orlando Normal susceptible strain, although TPP also reduced fipronil bioactivity slightly in two other strains. It is important to note that some studies with B. germanica use the LD_{50} dose of the susceptible strain on field-collected cockroaches, which would result in low mortality of field-collected strains and depressed fipronil mortality in two other strains. The only significant antagonism in our assays was caused by TPP in the Orlando Normal susceptible strain, although TPP also reduced fipronil bioactivity slightly in two other strains. It is important to note that some studies with B. germanica use the LD_{50} dose of the susceptible strain on field-collected cockroaches, which would result in low mortality of field-collected strains and depressed fipronil toxicity.

The lack of clear patterns in the involvement of detoxification enzymes in fipronil metabolism support the hypothesis that detoxification enzymes play a variable and relatively minor role in fipronil toxicity in the German cockroach, compared for example to the bed bug. Moreover, it is possible that intensive selection with pyrethroids, which has selected for relatively high resistance to pyrethroids (e.g., cypermethrin) in these populations, might have contributed to fipronil resistance. Hu et al. (2020) also suggested that high deltamethrin resistance in some cockroach strains could affect the performance of fipronil through upregulation of general cytochrome P450 monoxygenases that can detoxify both classes of compounds.

German cockroaches were likely pre-adapted for fipronil resistance, as indicated by the high correlation (i.e., cross-resistance) between dieldrin and fipronil resistance in the late 1990s in the United States (Holbrook et al. 2003) and Denmark (Kristensen et al. 2005). Recurrent treatments with dieldrin have selected for a mutation in the Rdl gene that results in a A302S/G substitution in Rdl in several phylogenetically diverse insect species (ffrench-Constant et al.)

Fig. 3. Nucleotide sequences of the TM2 region of the B. germanica Rdl gene, which includes the point mutation that results in the A302S substitution. (A) Representative sequences from the insecticide-susceptible Orlando Normal strain and five field-collected German cockroach populations were aligned against the reference sequence (MW267921.1), with the A302S region highlighted. (B) Representative direct sequencing chromatograms of homozygous susceptible (G/G) and homozygous resistant (T/T) cockroaches. The G-to-T point mutation site is shown within a box. MW267921.1 is the GenBank accession number for the B. germanica GABA-gated chloride channel complete cds (Jones et al. 2021).
Fipronil resistance in German cockroach populations has increased over the past three decades, but only marginally. Early findings in the late 1990s of fipronil resistance in the U.S. reported LD₉₀ resistance ratios (RR) <2, and follow-up studies in the 2000s found some populations with RR >17 (Table 1). Later reports, including recent findings, vary across studies and geographic locations, with RR values remaining low (1.0–8.7) in some populations (e.g., Liang et al. 2017, Wu and Appel 2017), while others increased appreciably as high as 38 (Gondhalekar et al. 2012, DeVries et al. 2019b, Lee et al. 2022a). The RR values we found (22.4–37.2) are thus consistent with the latter three papers given slight variations in methodology, reference strains used, and body mass of males.

The global patterns of resistance to fipronil appear to track the U.S. pattern. An early study showed highly variable RRs (1–15) among seven populations in Denmark (Kristensen et al. 2003); unfortunately, there are no recent follow-up studies of European B. germanica populations. In Asia, resistance to fipronil is generally lower than in the United States, likely because of later introduction of fipronil into the global indoor market, and slower adoption of baits and hence weaker selection pressure with fipronil. Among 24 field populations of B. germanica collected throughout Taiwan, fipronil RR values were 1.7–3.7, determined by applying fipronil to the surface and recording the LT₅₀, the time to 50% mortality (Hu et al. 2020).

The enigma of why fipronil resistance levels have remained low is particularly interesting because selection readily results in high levels of fipronil resistance in other insect species. In a common rice pest in Asia, the whitebacked planthopper (Sogatella furcifera Horváth, Hemiptera: Delphaciidae), artificial selection with fipronil for 11 generations doubled fipronil RRs up to 137.5 (Tang et al. 2010). Artificial selection with fipronil also increased the RR in the housefly, M. domestica, to 182 after five generations (Abbas et al. 2016) and in the cotton seed bug, Oecocerus balyaniennis Costa (Hemiptera: Lygaeidae), to 9,855 after 11 generations of selection (Wazir and Shad 2021). Moreover, fipronil RRs were 500 and 1,000 in two field-collected populations of the bed bug C. lectularius (González-Morales et al. 2021).

In contrast, when German cockroach populations were artificially selected with fipronil or fipronil-containing baits, RRs increased, but appeared to be somehow constrained, capping at 26 after five generations (Ang et al. 2013), 15.9 after 2 yr of selection (Ko et al. 2016), and 25 after 4 yr (Liang et al. 2017). Also, selection with a fipronil bait resulted in up to 4,000-fold increase in the RR to dieldrin but only 23.4-fold increase in the RR to fipronil (Ang et al. 2013), consistent with early observations that cross-resistance to fipronil was >2,000-fold lower than the level of resistance to dieldrin (Scott and Wen 1997). The limited effects of selection with fipronil appear to be evident in several other species. Populations of the brown planthopper, Nilaparvata lugens (Stål) (Hemiptera: Delphaciidae), selected with fipronil for eight generations, increased their RR to only 30.5 (Ling et al. 2009). Other examples come from field-collected arthropods that had been under intense selection with fipronil-containing pest management formulations. Field-collected cat flea populations, Ctenocephalides felis (Bouche) (Siphonaptera: Pulicidae), had low RRs, up to 2.2 (Rust et al. 2015), and fipronil RR values in brown dog tick populations, Rhipicephalus sanguineus Latreille (Ixodida: Ixodidae), topped at 13.8 (Becker et al. 2019).

It is likely that fipronil resistance in some arthropods, including B. germanica, carries significant fitness costs. For example, lower larval survival rate, lower adult emergence rate, lower copulation rate, lower fecundity and fewer offspring were associated with higher fipronil resistance in the brown planthopper (Ling et al. 2009, Zhang et al. 2016). Likewise, in the housefly, M. domestica, artificial selection with fipronil resulted in longer larval duration, lower pupal weight, lower fecundity, lower hatchability, lower number of the next generation larvae, lower intrinsic rate of population increases and lower biotic potential (Abbas et al. 2016). Other indirect evidence of significant fitness costs associated with fipronil resistance comes from observations that high resistance levels were not sustained when fipronil selection was discontinued in several insect species including planthoppers and houseflies. We therefore suspect that high fitness costs of fipronil resistance in the German cockroach might explain the moderate levels of resistance despite strong selection through several decades. Nevertheless, a study of fitness-related traits in B. germanica found no evidence of fitness costs; however, resistance to fipronil in the six field-collected strains ranged from 1.2- to 2.8-fold (Ang and Lee 2011), leaving this question unresolved. In contrast, resistance levels to pyrethroids are extremely high in B. germanica populations (Wei et al. 2001, DeVries et al. 2019b, Lee et al. 2022a, Scharf and Gondhalekar 2021), including in the three populations that we examined, and may be sustained for years after selection is discontinued (M.A.G-M and C.S., personal observations), suggesting lower fitness costs than with fipronil resistance. Overall, these patterns suggest potentially significant trade-offs between elevated levels of fipronil resistance in B. germanica and fitness-related traits. If so, this complex interaction has important implications for the continued use of fipronil in cockroach baits.

The mode of action of fipronil and its sulfone metabolite may also contribute to the stalled resistance in B. germanica. Together, fipronil and its bioactive metabolite are unique in binding with high affinity to three target sites in the insect central nervous system: GABA-gated chloride channels and two types of glutamate-gated chloride channels. Further, Zhao and Salgado (2010) suggested that there might be two subtypes of GABA receptors in the German cockroach. They also demonstrated that the Rdl mutation and resistance to dieldrin did not affect the sensitivity of GluCl receptors to fipronil sulfone. Generally, the evolution of target site-based resistance to
insecticides can be greatly slowed by ‘pyramiding’ two or more distinct toxins, as in pyramided Bt crops (Carrière et al. 2016). By binding multiple CNS targets, fipronil and fipronil sulfone may act as a combination of active ingredients that target different receptors, which is an important strategy of resistance management. However, much more information is needed about the levels of cross-resistance between fipronil and the sulfone and their differential affinities to different receptor sites.

Is Resistance to Fipronil Expected to Affect Its Field Efficacy?

There are no studies that quantitatively correlate fipronil resistance levels with the efficacy of fipronil baits in the field. We suspect that it is unlikely that 37-fold resistance to fipronil would affect the field efficacy of baits because of the high doses of fipronil ingested during bait consumption, which would overwhelm the relatively low resistance we observed. For example, Holbrook et al. (2003) supplemented rodent chow with fipronil and showed that concentrations much lower than in commercial baits killed all the resistant cockroaches (RR = 1.2 to >17). Moreover, based on consumption of 1 mg of bait per cockroach, Holbrook et al. (2003) estimated that each cockroach would ingest 100 ng of fipronil from a bait containing 0.01% fipronil (Maxforce FC gel – the standard in 2003, Bayer Environmental Science, Montvale, NJ). Ko et al. (2016) conducted more recent, but similar estimates. They determined the LD₉₀ of fipronil in susceptible males to be 3.18 ng per male (2.67 ng in the present study). If a susceptible male ingested 1.8 mg of bait containing 0.05% fipronil (Maxforce FC Magnum, Bayer), it would consume 900 ng of fipronil, or 370-fold the LD₉₀. Because fipronil is more active at lower levels than most other insecticides, the high-dose strategy inherent in gel bait formulations, and especially those containing fipronil, could overcome the highest fipronil resistance reported thus far in any German cockroach population.

However, the foregoing estimates assume highly palatable baits that are consumed in large quantities and thus deliver a high dose of fipronil. Behavioral traits such as avoidance of the bait (e.g., glucose aversion) or physical changes in the formulation (aging, repellency) could result in lower bait consumption and compromise control efforts. Glucose aversion has become more prevalent since it was discovered 3 decades ago (Silverman and Bieman 1993, Wang et al. 2004, Wada-Katsumata et al. 2013). Moreover, recent research showed that cockroach saliva rapidly degrades various sugars, releasing glucose, which interrupts feeding by glucose-averse cockroaches (Wada-Katsumata and Schal 2021, Wada-Katsumata et al. 2022). Thus, bait formulations that contain sugars, not just glucose, would deter glucose-averse cockroaches. We underscore that bait palatability, resistance to the active ingredient, and expected performance in the field are intricately linked. Highly palatable baits deliver enough fipronil to overcome ~30–40-fold resistance that we observe in the field. However, less palatable baits would deliver less fipronil, and ~30–40-fold resistance might offer ample protection to field cockroaches.

The recent introduction of residual formulations of fipronil might have a similar effect. In 2019, fipronil was approved in the United States for use in residual sprays (0.65%, Fipronil-Plus-C, EPA Reg. No. 55431-15; maximum allowed fipronil concentration 0.0076%) for controlling crawling insects indoors, including cockroaches. Residual sprays that expose insects to much lower doses than those found in baits might nullify the advantages of the high-dose approach with baits, and even the moderate ~30–40-fold present-day fipronil resistance might protect cockroaches from residual fipronil formulations. A case in point may be the dual use of indoxacarb in baits and spray formulations. Indoxacarb has been used in baits since 2006 and in spray formulations since 2010. The increase in resistance and control issues over the years may be due to the dual use, which compromises the high-dose strategy (Gondhalekar et al. 2013).

Conclusion

The accumulated evidence on fipronil resistance in B. germanica suggests that target site insensitivity in Rdl contributes to low-level resistance to fipronil, and metabolic detoxification processes are highly variable across populations and contribute marginally to fipronil resistance. The metabolic mechanisms might be related to low fipronil cross-resistance with pyrethroids, which select for significant upregulation of a wide range of P450 genes. Our results suggest that combining fipronil with PBO or DEF could reduce fipronil resistance in the most resistant populations. However, this might not be necessary if fitness costs associated with fipronil resistance, perhaps related to ensuring proper functioning of the GABA-gated channels and glutamate-gated chloride channels, will prevent the emergence of highly resistant German cockroach populations. In practical cockroach control, lower fitness of resistant cockroaches would lend strong support for a resistance management program based on the practice of rotation of active ingredients, because populations are expected to quickly recover sensitivity to fipronil when fipronil pressure is withdrawn. Highly palatable baits can deliver high doses of fipronil that can overcome present-day resistance levels. However, less palatable baits (e.g., poor quality, rancid), and taste aversions in cockroaches (e.g., glucose-aversion) would result in less fipronil ingested, and ~30–40-fold resistance to fipronil might protect cockroaches from such baits.

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