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Pervasive Resistance to Pyrethroids in German Cockroaches (Blattodea: Ectobiidae) Related to Lack of Efficacy of Total Release Foggers

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Abstract

Despite limited efficacy data, do-it-yourself (DIY) insecticide products often promise low-cost alternatives to professional pest control. Total release foggers (TRFs, 'bug bombs'), which are prominent DIY products, were recently shown to be ineffective at reducing German cockroach (*Blattella germanica* L.) infestations, in contrast to highly effective baits. However, the reason(s) for TRF failure remain unknown. Therefore, we investigated insecticide resistance of apartment-collected cockroaches from homes where TRFs failed. In topical (direct) application assays, resistance to cypermethrin (a common active ingredient in TRFs) was 202 ± 33 times that of a laboratory insecticide-susceptible population (based on LD_{50} ratios), while resistance to fipronil, a common bait active ingredient, was considerably lower at 14 ± 2 times that of the laboratory insecticide-susceptible population. The addition of PBO, a P450 inhibitor that synergizes pyrethroids, enhanced the efficacy of cypermethrin, but only at high doses of cypermethrin. Additionally, >96% of screened cockroaches possessed at least one copy of the L993F mutation in the voltage-gated sodium channel, known to confer resistance to pyrethroids (knockdown resistance, *kdr*). Because TRF treatments killed insecticide-susceptible sentinel cockroaches but failed to kill apartment-collected cockroaches, these results suggest that pyrethroid resistance is a major factor contributing to the failure of TRFs. Multiple mechanisms of resistance, including metabolic detoxification of the pyrethroids and *kdr* mutations that confer target-site insensitivity, suggest that TRFs would lack efficacy against German cockroaches in residential settings, where high levels of pyrethroid resistance have been documented globally.

Key words: cypermethrin, do-it-yourself pest control, *kdr*, topical application

Cockroach control continues to rely heavily on residual applications of broad-spectrum insecticides, with varying degrees of efficacy related to the active ingredient, formulation, application techniques, and most importantly the rapid evolution of insecticide resistance. Despite the documented efficacy of gel baits, some of which are do-it-yourself (DIY) products, consumers continue to rely heavily on a variety of residual insecticide formulations, including aerosol sprays and total release foggers (TRFs). Total release foggers are a common DIY insecticidal product, used to control a variety of indoor and structural pests, but despite their ubiquity, recent reports have shown TRFs to be ineffective at controlling bed bugs, *Cimex lectularius* L. (Hemiptera: Cimicidae) (Jones and Bryant 2012) and German cockroaches, *Blattella germanica* (L.) (DeVries et al. 2019). In the case of the German cockroach, this is particularly troubling because of the significant effects this species can have on human health, namely exacerbation of asthma through the production of allergens (Rosenstreich et al. 1997, Eggleston et al. 1998, Gore and Schal 2007), and transmission of pathogenic microorganisms (Brenner 1995, Ahmad et al. 2011). Additionally, these products distribute large amounts of pesticide residues on all horizontal surfaces throughout the discharge site, including areas where pests such as cockroaches and bed bugs are unlikely to encounter them (Keenan et al. 2010, DeVries et al. 2019). Despite these risks, the mechanisms responsible for TRF failure remain unclear.

Although multiple mechanisms can result in failure of insecticidal products, we suspect that resistance to pyrethroids plays a major role in the failure of TRFs (DeVries et al. 2019). German cockroach resistance to pyrethroids is highly pervasive and has persisted for over 30 yr (Cochran 1989, Atkinson et al. 1991, Wei et al. 2001, Holbrook et al. 2003, Chai and Lee 2010, Fardisi et al. 2017, Wu and Appel 2017). The continued use of residual spray formulations, TRFs, and aerosol sprays has made pyrethroids largely ineffective compared to insecticide baits (Miller and Meek 2004, Nalyanya et al. 2009). However, most studies of resistance in the German cockroach have not provided direct links between treatment failures and insecticide resistance, rather relying on local, regional, or even global patterns of resistance prevalence. In our previous study documenting the lack of efficacy of TRFs (DeVries et al. 2019), we showed that >62% of sentinel cockroaches, collected in each apartment and exposed in open-top cages to the TRF aerosols, survived the treatment. In contrast, <10% of Orlando Normal susceptible cockroaches survived, suggesting that pyrethroid resistance may be a significant barrier to TRF efficacy. Therefore, we designed a study to assess insecticide resistance in cockroaches collected from a subset of the homes where TRFs failed (DeVries et al. 2019). Resistance was characterized by topical application of insecticides and molecular characterization of knockdown (*kdr*) mutations. These findings, along with our previous results on the ineffectiveness of TRFs and their extensive insecticide contamination of indoor residential surfaces, provide compelling support for suspending their use for German cockroach control.

Materials and Methods

Cockroach Populations

In a previous study, where TRFs were evaluated and found to be ineffective (DeVries et al. 2019), German cockroaches were collected from a subset of the infested apartments, representing five different apartment complexes in Raleigh, NC. All collections were made under Institutional Review Board approval (NC State University # 1459). Briefly, prior to TRF treatment cockroaches were collected using a modified Eureka Mighty-Mite 7.0-ampere vacuum cleaner (Eureka Company, Charlotte, NC). Live cockroaches were collected into a mesh-lined plastic tube attached to the distal end of the vacuum's extension tube. Cockroaches were reared for one generation to establish populations sufficient for testing. In addition, an insecticide-susceptible population (Orlando Normal = American Cyanamid strain), collected >60 yr ago in a Florida apartment, was used to determine resistance ratios (RR).

All cockroach populations were reared in plastic bins (20 × 15 × 10 cm^3) at 27°C and 40–70% RH on a 12:12 (L:D) h photoperiod. Water and food (rodent chow, LabDiet 5001, PMI Nutrition International, Brentwood, MO) were provided ad libitum, and cardboard egg cartons were provided as harborage. Only healthy adult males (unknown age) were used for all experiments.

Topical Applications

The lethal dose that killed 50% of each population (LD_{50}) was determined by topical application of two insecticides: cypermethrin (>90% purity; a pyrethroid representative of TRF products; Sigma-Aldrich, St. Louis, MO) and fipronil (>89% purity; representative of over-the-counter gel baits; Sigma-Aldrich). Adult male cockroaches were briefly anesthetized (CO_2), separated into groups of 10 in round plastic Petri dishes (90 mm × 15 mm) and 1 µl of acetone containing technical grade insecticide was applied to the ventral side between the coxae using a 50 µl syringe in a repeating dispenser (Hamilton Company, Reno, NV). Dilutions ranged from 0 ng (acetone-only control) to 50 µg (cypermethrin) or 300 ng (fipronil), based on preliminary toxicity assays. Mortality was assessed 24 h (cypermethrin) or 48 h (fipronil) post-application, with moribund cockroaches (those unable to right themselves or exhibiting uncoordinated movement) considered dead.

Synergism With Piperonyl Butoxide

The pyrethroid synergist piperonyl butoxide (PBO, >98% purity; Sigma-Aldrich) was tested for its effects on cypermethrin toxicity using two discriminating doses—0.5 and 5 µg of cypermethrin per cockroach. These doses were selected to represent 10 and 100 times the LD_{50} of the Orlando Normal (insecticide-susceptible) population. We used the same protocol as above, except that each insect was topically treated with 100 µg of PBO in 1 µl acetone 1 h before topically applying either 0.5 or 5 µg of cypermethrin. Mortality was assessed 24 h later.

Knockdown Resistance

Knockdown resistance (*kdr*) mutations were evaluated in 10 cockroaches from each of 10 apartments and from 20 cockroaches from the insecticide-susceptible Orlando Normal population. Total RNA (RNeasy kit, Qiagen, Valencia, CA) was extracted from individual whole insects as per the manufacturer's protocol. First-strand cDNA was synthesized from 5 µl of RNA using Tetro cDNA synthesis kit (Bioline, Taunton, MA). The cDNA was used to amplify four different regions of the para-sodium channel involved in *kdr* resistance (Dong 1997, Liu et al. 2000) using site-specific primers (listed in Table 1). The PCR reactions were conducted in 25 µl reaction mix comprising 12.5 µl 2× TopTaq Master Mix (Qiagen), 0.5 µl each of forward and reverse primers at 10 µM concentration, 1 µl of cDNA template, and 10.5 µl RNase-free water. The PCR conditions used for amplification of C764R and L993F targets were: 95°C for 3 min, followed by 40 cycles at 94°C for 45 s, the respective annealing

Table 1. Primers used to assess *kdr* mutations in apartment-collected German cockroach populations, based on Liu et al. (2000)

Point mutation[a]	bp change[b]	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Annealing temperature (°C)	Amplicon size (bp)
E434K	G^{1300} to A	CCCTGGCATATGCTGTTCTT	CTGCCAGTTTATCCGCTTGT	48	210
C764R	T^{2290} to C	CGACAGACGAAGATGACGAG	ACCACAATGCAAAGGGTGAT	57	190
L993F	G^{2979} to C	GTTGGAGACTGGTCCTGCAT	CGCGCTACGTTTTATCCAGT	55	220
P1880L	C^{5639} to T	TTGACGGATGATGACTACGATATG	AAAGAAGTCCTTCGTCAAAGCAT	56	231

[a]The point mutation refers to the amino acid position in the translated protein. The letter at left denotes the wild-type amino acid, whereas the letter at right denotes the *kdr* variant amino acid.

[b]bp change identifies the position of the base change in the translated sequence.

temperature (Table 1) for 45 s, 72°C for 45 s followed by a final extension at 72°C for 5 min. A 38-cycle PCR with similar conditions was used to amplify P1880L. For E434K, a 10-cycle touchdown PCR was conducted: 95°C for 3 min, 94°C for 45 s, with annealing temperatures lowering from 60°C to 55°C (lowered by 0.5°C each cycle) for 45 s, 72°C for 45 s, followed by 40 cycle of PCR at the annealing temperature of 48°C. The PCR products were tested on 2% agarose gels for the presence of correct size fragments (Table 1). The amplicons were purified (Qiaquick PCR purification kit, Qiagen) and directly sequenced (Sanger) at the Genomic Sciences Laboratory (North Carolina State University, Raleigh, NC). Poor-quality sequences were removed from the analysis. The sequences were multiple aligned and analyzed for the presence of single-nucleotide polymorphisms (SNPs) of interest using reference genes (U73584) from NCBI.

Data Analyses

Probit analysis was used to determine the LD_{50} for the insecticides cypermethrin and fipronil for the laboratory and apartment-collected cockroach populations. Resistance ratios were calculated by dividing the LD_{50} of each apartment-collected cockroach population by the LD_{50} of the Orlando Normal insecticide-susceptible population. The effect of the pyrethroid synergist PBO on cypermethrin toxicity (two doses) was evaluated using a Student's *t*-test at each cypermethrin dose on arc-sine square root transformed percent mortality. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC).

Results

Topical Applications

All apartment-collected cockroaches were highly resistant to cypermethrin (Table 2). The average resistance ratio relative to the Orlando Normal insecticide-susceptible population was 202 ± 33 (SEM)-fold. The average slope for all probit lines for the apartment-collected populations was 2.60 ± 0.32, shallower in comparison to a slope of 5.90 ± 0.48 for the susceptible population, indicating greater tolerance over a broad range of cypermethrin doses.

Apartment-collected cockroaches were also moderately resistant to fipronil (Table 3). Their average resistance ratio compared to the susceptible Orlando Normal population was 14 ± 2, and the average slope of their probit lines was 3.95 ± 0.24, compared to 15.32 ± 2.40 for the laboratory population.

Synergism With PBO

Piperonyl butoxide significantly enhanced the toxicity of topically applied cypermethrin at both 0.5 µg (t_{14} = 3.97, P = 0.0014) and 5 µg (t_{14} = 11.12, P < 0.0001) (Table 4). However, even when synergized with PBO, 0.5 µg cypermethrin (~10 times the LD_{50} of the susceptible population) resulted in minimal mortality (7.5 ± 2.4%) in apartment-collected populations. These results indicate substantial metabolic resistance to cypermethrin across all apartment-collected German cockroaches.

Knockdown Resistance

Four different regions of the para-sodium channel were analyzed in 10 apartment-collected and a laboratory population for four different mutations involved in *kdr* resistance. The L993F *kdr* mutation, known to confer pyrethroid resistance, was prevalent in all cockroach populations, with 96% of all apartment-collected cockroaches possessing at least one copy of this mutation (i.e., 'resistant' genotype) and 77% being homozygous for this mutation (Table 5). Only 4% of all apartment-collected cockroaches lacked this mutation (i.e., exhibited the 'susceptible' genotype), and these individuals came from only three populations.

In addition, E434K and C764R mutations, known to be associated with *kdr* resistance (Liu et al. 2000), were found at low frequency (<10%) in two populations (Table 6). We detected no P1880L mutations (Table 6).

Discussion

German cockroaches collected from homes where TRFs failed, were highly resistant to cypermethrin. Therefore, given that pyrethroids represent the only class of active ingredient found in TRFs, it is not surprising that these products failed. Resistance to pyrethroids has

Table 2. Resistance of apartment-collected German cockroach populations to topically applied cypermethrin

Population[a]	n	LD_{50} (µg per male[b])	LD_{50} 95% CI[c]	Slope ± SE	χ^2 (df)	RR[d]
Orlando Normal[e]	600	0.049	0.046–0.051	5.90 ± 0.48	11.11 (11)	–
C45	180	17.025	12.90–24.50	2.08 ± 0.36	0.02 (3)	347
C50	180	11.568	9.76–15.11	3.89 ± 0.83	0.49 (3)	236
F2215	87	4.657	2.67–5.72	4.52 ± 1.41	<0.01 (1)	95
F2225	90	2.874	1.79–4.26	1.80 ± 0.32	2.50 (1)	59
P511	120	13.081	9.34–17.87	2.23 ± 0.47	0.03 (1)	267
R2919	180	6.905	5.02–8.80	2.44 ± 0.48	0.87 (3)	141
R3011	150	15.361	11.50–22.10	1.95 ± 0.35	0.09 (2)	313
S1224	120	10.653	6.96–15.18	1.81 ± 0.36	0.48 (1)	217
S1320	150	7.213	5.37–9.04	2.67 ± 0.54	2.46 (2)	147
Average[f]		9.926	–	2.60	–	202

[a]Cockroach populations are represented from each of five apartment complexes in Raleigh, NC. Letters designate unique apartment complexes, and number designates apartment (population).

[b]The average mass of *B. germanica* males is 50 mg, hence multiply by 20 to obtain approximate µg/g body mass.

[c]CI: confidence interval.

[d]RR: resistance ratio = LD_{50} of apartment population/LD_{50} of the Orlando Normal population.

[e]Orlando Normal is a standard insecticide-susceptible population.

[f]Averages for the nine apartment-collected populations.

Table 3. Resistance of apartment-collected German cockroach populations to topically applied fipronil

Population[a]	n	LD_{50} (ng per male[b])	LD_{50} 95% CI[c]	Slope ± SE	χ^2 (df)	RR[d]
Orlando Normal[e]	180	2.99	2.87–3.11	15.32 ± 2.40	3.93 (4)	–
C45	150	47.48	37.30–61.03	3.54 ± 0.55	3.43 (3)	16
C50	90	33.20	23.68–42.37	3.83 ± 0.94	<0.01 (1)	11
F2215	89	69.00	54.93–85.09	5.07 ± 0.98	<0.01 (1)	23
P511	120	38.74	30.68–49.13	3.90 ± 0.68	0.29 (2)	13
R2919	89	16.62	11.84–27.85	2.98 ± 0.60	1.57 (1)	6
S1224	90	42.70	33.19–54.12	4.06 ± 0.82	<0.01 (1)	14
S1320	90	50.36	39.96–63.41	4.30 ± 0.79	<0.01 (1)	17
Average[f]		42.59	–	3.95	–	14

[a]Cockroach populations are represented from each of five apartment complexes in Raleigh, NC. Letters designate unique apartment complexes, and number designates apartment (population).

[b]The average mass of *B. germanica* males is 50 mg, hence multiply by 20 to obtain approximate µg/g body mass.

[c]CI: confidence interval.

[d]RR: resistance ratio = LD_{50} of apartment-collected population/LD_{50} of laboratory population.

[e]Orlando Normal is a standard insecticide-susceptible population.

[f]Averages for the seven apartment-collected populations.

Table 4. Effect of the insecticide synergist PBO on cypermethrin toxicity

	0.5 µg cypermethrin per male (10-fold the LD_{50} of the susceptible population)				5 µg cypermethrin per male (100-fold the LD_{50} of the susceptible population)			
	– PBO		+ PBO[a]		– PBO		+ PBO	
Population[b]	n	Mortality (%)[c]	n	Mortality (%)	n	Mortality (%)	n	Mortality (%)
C45	30	0.0	30	10.0	30	13.3	30	96.7
C50	30	0.0	30	0.0	30	10.0	30	100.0
F2215	30	0.0	30	6.7	27	55.6	30	100.0
P511	30	0.0	30	20.0	30	0.0	30	96.7
R2919	30	0.0	30	3.3	30	33.3	30	100.0
R3011	30	0.0	30	0.0	30	16.7	30	96.7
S1224	30	0.0	30	13.3	30	3.3	30	100.0
S1320	30	0.0	30	6.7	30	26.7	30	100.0
Average[d]		0.0 (±0.0)		7.5 (±2.4)*[e]		19.9 (±6.4)		98.8 (±0.6)*

[a]PBO (100 µg) was applied 1 h prior to cypermethrin application.

[b]Cockroach populations are represented from each of five apartment complexes in Raleigh, NC. Letters designate unique apartment complexes, and number designates apartment (population).

[c]Percent mortality was determined after 24 h.

[d]Average percent mortality for the eight apartment-collected populations (±SEM).

[e]Significance between treatments with and without the addition of PBO is indicated by an * (Student's *t*-test).

been well documented in many populations of German cockroaches around the globe (Cochran 1989, 1995; Atkinson et al. 1991; Wei et al. 2001; Chai and Lee 2010; Fardisi et al. 2017; Wu and Appel 2017), indicating that TRFs will likely be ineffective regardless of the geographic location where they are used. Additionally, our results indicate that pyrethroid selection pressure has not declined over time, with resistance to cypermethrin averaging >200-fold compared to a cypermethrin-susceptible population.

To further elucidate the mechanisms responsible for pyrethroid resistance, we screened populations for both target-site insensitivity (*kdr* mutations) and metabolic detoxification. Prevalence of the L993F *kdr* mutation, known to confer resistance to pyrethroids (Dong 1997, Dong et al. 1998, Liu et al. 2000), was high among all populations screened, providing compelling evidence for why TRFs were ineffective in homes. Valles (2004) showed that selection with cypermethrin alone could increase the prevalence of the L993F *kdr* mutation. However, the populations selected by Valles (2004) did not have significant increases in enzymatic activity, and the resistance ratio compared to an insecticide-susceptible population was only 35 times higher, suggesting that multiple resistance mechanisms are needed to reach resistance ratios such as those observed in the current study (e.g., >59-fold). Not surprisingly, all populations that we screened exhibited higher mortality when treated with cypermethrin and PBO, indicating that detoxification enzymes (i.e., P450s) play a prominent role in pyrethroid resistance in these populations (Scott et al. 1990). However, even with the addition of PBO, German cockroaches remained moderately resistant to cypermethrin, with a cypermethrin dose 10 times higher than the LD_{50} for the insecticide-susceptible population resulting in only 7.5% mortality in apartment-collected populations (Table 4). Taken together with the findings that PBO-containing TRFs neither reduced German cockroach populations nor killed sentinel cockroaches (DeVries et al. 2019), it is clear that multiple resistance mechanisms contribute to TRF failure. Nevertheless, we did not directly determine the relative importance of each resistance mechanism, and thus further work is needed to understand the dynamic interactions among various resistance mechanisms. In addition, other synergists should be screened in a similar fashion to determine if the expression or activity of other detoxification enzymes such as esterases, glutathione *S*-tranferases, and carboxylesterases increases in homes where synergists are used (e.g., Valles and Strong 2001).

In parallel, we screened all cockroach populations for resistance to fipronil, a common ingredient of gel baits. Although all apartment-collected cockroach populations were moderately resistant (6- to 23-fold) to fipronil, baits containing fipronil were highly effective at reducing German cockroach populations in direct and simultaneous comparisons with TRFs (70–93% reduction after 1 mo, DeVries et al. 2019). The formulation used to deliver insecticides to insects plays a significant role in the ultimate impact of insecticide resistance on the efficacy of pest control. Baits have two key advantages over spray and TRF formulations: 1) a considerably smaller dose of an ingested insecticide can cause mortality than the dose required by contact (Sierras and Schal 2017), and 2) much higher doses are formulated into baits than sprays relative to the LD_{50} dose (Holbrook et al. 2003, Ko et al. 2016). These features of baits, coupled with proper bait rotations (i.e., active ingredient with different modes of action), and careful attention to whether baits are accepted by cockroaches (e.g., Wada-Katsumata et al. 2013) can effectively extend the range of resistance that can be tolerated for baits.

It is important also to note that despite the use of baits containing fipronil for >15 yr, resistance has remained moderate and has increased only slightly over this time. Early reports, either before fipronil was used for cockroach control or shortly after, documented average resistance ratios of 1.4-fold (range: 1.0–1.8) across 5 unselected populations collected in the United States (Scott and Wen 1997), 5.1-fold (range: 1.0–15.0) in 7 populations from Denmark (Kristensen et al. 2005), and up to 17-fold (average not provided) in 29 populations collected throughout North Carolina (Holbrook et al. 2003). More recent reports show this ratio at 2.0-fold (range = 1.0–10.0) in 21 populations in Singapore (Chai and Lee 2010), 5.3-fold (range = 2.0–7.6) in 6 populations from Franklin County, NC (Wu and Appel 2017), 14-fold (range = 5.6–23.1) in 7 populations from Wake County, NC (current study), and 36-fold in a single population from Gainesville, FL (Gondhalekar and Scharf 2012). This trend is particularly notable, because despite greater adoption of baits in German cockroach control (PCT 2016), fipronil resistance has not escalated as would be expected from such strong selection. It should be noted that there is currently no empirically derived relationship between the resistance ratio to bait active ingredients and the efficacy of cockroach control. Cochran (1995) suggested resistance ratios greater than 20-fold could compromise control efficacy, but no empirical data have been collected to test this

Table 5. Expressed frequencies of the L993F *kdr* mutation in *B. germanica*

	L993F			
Population[a]	n	S/S[b]	S/R[c]	R/R[d]
Orlando Normal[e]	20	1.00	0.00	0.00
C45	8	0.00	0.13	0.87
C50	9	0.00	0.00	1.00
F2225	10	0.10	0.10	0.80
F2289	9	0.11	0.22	0.67
P511	8	0.00	0.00	1.00
P619	10	0.20	0.30	0.50
R2919	10	0.00	0.50	0.50
R3011	8	0.00	0.38	0.62
S1224	10	0.00	0.20	0.80
S1320	10	0.00	0.10	0.90
Average[f]		0.04	0.19	0.77

[a]Cockroach populations are represented from each of five apartment complexes in Raleigh, NC. Letters designate unique apartment complexes, and number designates apartment (population).

[b]S/S = proportion homozygous wild-type (considered pyrethroid-susceptible).

[c]S/R = proportion heterozygous.

[d]R/R = proportion homozygous for the *kdr* mutation (considered to confer resistance to pyrethroids).

[e]Orlando Normal is a standard insecticide-susceptible population.

[f]Average of all 10 apartment-collected populations.

Table 6. Expressed frequencies of *kdr* mutations E434K, C764R, and P1880L

	E434K				C764R				P1880L			
Population[a]	n	S/S[b]	S/R[c]	R/R[d]	n	S/S	S/R	R/R	n	S/S	S/R	R/R
Orlando Normal[e]	10	1.00	0.00	0.00	10	1.00	0.00	0.00	10	1.00	0.00	0.00
C45	10	1.00	0.00	0.00	10	1.00	0.00	0.00	9	1.00	0.00	0.00
C50	10	1.00	0.00	0.00	10	1.00	0.00	0.00	9	1.00	0.00	0.00
F2225	10	1.00	0.00	0.00	10	1.00	0.00	0.00	9	1.00	0.00	0.00
F2289	9	1.00	0.00	0.00	9	1.00	0.00	0.00	9	1.00	0.00	0.00
P511	10	1.00	0.00	0.00	10	1.00	0.00	0.00	10	1.00	0.00	0.00
P619	10	1.00	0.00	0.00	10	1.00	0.00	0.00	7	1.00	0.00	0.00
R2919	10	1.00	0.00	0.00	10	1.00	0.00	0.00	10	1.00	0.00	0.00
R3011	10	0.90	0.10	0.00	10	1.00	0.00	0.00	10	1.00	0.00	0.00
S1224	10	0.90	0.00	0.10	10	0.90	0.00	0.10	10	1.00	0.00	0.00
S1320	10	1.00	0.00	0.00	10	1.00	0.00	0.00	9	1.00	0.00	0.00
Average[f]		0.98	0.01	0.01		0.99	0.00	0.01		1.00	0.00	0.00

[a]Cockroach populations are represented from each of five apartment complexes in Raleigh, NC. Letters designate unique apartment complexes, and number designates apartment (population).

[b]S/S = proportion homozygous wild-type (considered pyrethroid-susceptible).

[c]S/R = proportion heterozygous.

[d]R/R = proportion homozygous for the *kdr* mutation (considered to confer resistance to pyrethroids).

[e]Orlando Normal is a standard insecticide-susceptible population.

[f]Average of all 10 apartment-collected populations.

threshold and more work is needed in this area. Future work should characterize the genetic mechanisms of fipronil resistance, its impact on fitness, and the stability of resistance when selection is relaxed.

Overall then, two related factors contribute to the lack of efficacy of TRF products: the use of pyrethroids as active ingredients and their broadcast aerosolization. As noted above, resistance to cypermethrin (and other pyrethroids) is pervasive and high among these and other German cockroach populations, which would require high application rates to deliver high doses to cockroaches. A typical application rate of 5 liters per 100 m^2 of a 0.2% cypermethrin wettable powder formulation would deliver 100 mg AI/m^2; treatment with a 0.5% suspension concentrate preparation would result in 250 mg AI/m^2 (Wickham 1995). Yet the highest concentration of cypermethrin we detected on surfaces in TRF-treated homes immediately after their deployment was <8 mg/m^2 (DeVries et al. 2019). Thus, although the overall discharge of insecticide from TRF devices may be high, the aerosol is broadly deposited on all horizontal surfaces, resulting in cypermethrin residues that are 12- to 31-fold less than in typical crack-and-crevice spray applications. Taken together, the dilution of TRF active ingredients during broad aerosolization and the high and pervasive resistance to pyrethroids documented in German cockroaches do not bode well for current TRF products. Given the extensive global use of pyrethroids indoors and high resistance of German cockroaches to pyrethroids, it is highly likely that the pattern we documented in multiple independent apartment complexes in North Carolina is representative of the broad pattern in the United States and likely globally. If so, our demonstration of the ineffectiveness of TRFs, prevalent resistance to pyrethroids, the unavoidable contamination of indoor residential surfaces resulting from the proper deployment of TRFs, and injuries and home fires from misuse of TRFs should be compelling arguments for the discontinuation of TRF use for German cockroach control.

In conclusion, this report is notable for empirically demonstrating a direct link between product failure in multiple apartments and insecticide resistance due to multiple resistance mechanisms in the same German cockroach populations. It is more common to indirectly associate declining performance of insecticide products in the indoor environment with broad patterns of resistance, often at regional or even global scales, rather than with the same populations where pest control failed.

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References Cited

Ahmad, A., A. Ghosh, C. Schal, and L. Zurek. 2011. Insects in confined swine operations carry a large antibiotic resistant and potentially virulent enterococcal community. BMC Microbiol. 11: 23.

Atkinson, T. H., R. W. Wadleigh, P. G. Koehler, and R. S. Patterson. 1991. Pyrethroid resistance and synergism in a field strain of the German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 84: 1247–1250.

Brenner, R. 1995. Economics and medical importance of German cockroaches, pp. 77–92. *In* M. Rust, J. Owens, and D. Reierson (eds.), Understanding and controlling the German cockroach. Oxford University Press, New York.

Chai, R. Y., and C. Y. Lee. 2010. Insecticide resistance profiles and synergism in field populations of the German cockroach (Dictyoptera: Blattellidae) from Singapore. J. Econ. Entomol. 103: 460–471.

Cochran, D. G. 1989. Monitoring for insecticide resistance in field-collected strains of the German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 82: 336–341.

Cochran, D. 1995. Insecticide resistance, pp. 150–290. *In* M. K. Rust, J. M. Owens, and D. A. Reierson (eds.), Understanding and controlling the German cockroach, 10th ed. Oxford University Press, New York.

DeVries, Z. C., R. G. Santangelo, J. Crissman, R. Mick, and C. Schal. 2019. Exposure risks and ineffectiveness of total release foggers (TRFs) used for cockroach control in residential settings. BMC Public Health. 19: 96.

Dong, K. 1997. A single amino acid change in the para sodium channel protein is associated with knockdown-resistance (*kdr*) to pyrethroid insecticides in German cockroach. Insect Biochem. Mol. Biol. 27: 93–100.

Dong, K., S. M. Valles, M. E. Scharf, B. Zeichner, and G. W. Bennett. 1998. The knockdown resistance (*kdr*) mutation in pyrethroid-resistant German cockroaches. Pestic Biochem. Physiol. 60: 195–204.

Eggleston, P. A., D. Rosenstreich, H. Lynn, P. Gergen, D. Baker, M. Kattan, K. M. Mortimer, H. Mitchell, D. Ownby, R. Slavin, *et al.* 1998. Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma. J. Allergy Clin. Immunol. 102: 563–570.

Fardisi, M., A. D. Gondhalekar, and M. E. Scharf. 2017. Development of diagnostic insecticide concentrations and assessment of insecticide susceptibility in German cockroach (Dictyoptera: Blattellidae) field strains collected from public housing. J. Econ. Entomol. 110: 1210–1217.

Gondhalekar, A. D., and M. E. Scharf. 2012. Mechanisms underlying fipronil resistance in a multiresistant field strain of the German cockroach (Blattodea: Blattellidae). J. Med. Entomol. 49: 122–131.

Gore, J. C., and C. Schal. 2007. Cockroach allergen biology and mitigation in the indoor environment. Annu. Rev. Entomol. 52: 439–463.

Holbrook, G. L., J. Roebuck, C. B. Moore, M. G. Waldvogel, and C. Schal. 2003. Origin and extent of resistance to fipronil in the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). J. Econ. Entomol. 96: 1548–1558.

Jones, S. C., and J. L. Bryant. 2012. Ineffectiveness of over-the-counter total-release foggers against the bed bug (Heteroptera: Cimicidae). J. Econ. Entomol. 105: 957–963.

Keenan, J. J., J. H. Ross, V. Sell, H. M. Vega, and R. I. Krieger. 2010. Deposition and spatial distribution of insecticides following fogger, perimeter sprays, spot sprays, and crack-and-crevice applications for treatment and control of indoor pests. Regul. Toxicol. Pharmacol. 58: 189–195.

Ko, A. E., D. N. Bieman, C. Schal, and J. Silverman. 2016. Insecticide resistance and diminished secondary kill performance of bait formulations against German cockroaches (Dictyoptera: Blattellidae). Pest Manag. Sci. 72: 1778–1784.

Kristensen, M., K. K. Hansen, and K. M. Jensen. 2005. Cross-resistance between dieldrin and fipronil in German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 98: 1305–1310.

Liu, Z., S. M. Valles, and K. Dong. 2000. Novel point mutations in the German cockroach para sodium channel gene are associated with knockdown resistance (*kdr*) to pyrethroid insecticides. Insect Biochem. Mol. Biol. 30: 991–997.

Miller, D. M., and F. Meek. 2004. Cost and efficacy comparison of integrated pest management strategies with monthly spray insecticide applications for German cockroach (Dictyoptera: Blattellidae) control in public housing. J. Econ. Entomol. 97: 559–569.

Nalyanya, G., J. C. Gore, H. M. Linker, and C. Schal. 2009. German cockroach allergen levels in North Carolina schools: comparison of integrated pest management and conventional cockroach control. J. Med. Entomol. 46: 420–427.

PCT. 2016. State of the cockroach market. Pest Control Technology. Valley View, OH.

Rosenstreich, D. L., P. Eggleston, M. Kattan, D. Baker, R. G. Slavin, P. Gergen, H. Mitchell, K. McNiff-Mortimer, H. Lynn, D. Ownby, *et al.* 1997. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N. Engl. J. Med. 336: 1356–1363.

Scott, J. G., and Z. Wen. 1997. Toxicity of fipronil to susceptible and resistant strains of German cockroaches (Dictyoptera: Blattellidae) and house flies (Diptera: Muscidae). J. Econ. Entomol. 90: 1152–1156.

Scott, J. G., D. G. Cochran, and B. D. Siegfried. 1990. Insecticide toxicity, synergism and resistance in the German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 83: 1698–1703.

Sierras, A., and C. Schal. 2017. Comparison of ingestion and topical application of insecticides against the common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae). Pest Manag. Sci. 73: 521–527.

Valles, S. M. 2004. Effects of cypermethrin selection on expression of insecticide resistance mechanisms in the German cockroach (Blattaria: Blattellidae). J. Entomol. Sci. 39: 84–93.

Valles, S. M., and C. A. Strong. 2001. A microsomal esterase involved in cypermethrin resistance in the German cockroach, *Blattella germanica*. Pest. Biochem. Physiol. 71: 56–67.

Wada-Katsumata, A., J. Silverman, and C. Schal. 2013. Changes in taste neurons support the emergence of an adaptive behavior in cockroaches. Science. 340: 972–975.

Wei, Y., A. G. Appel, W. J. Moar, and N. Liu. 2001. Pyrethroid resistance and cross-resistance in the German cockroach, *Blattella germanica* (L). Pest Manag. Sci. 57: 1055–1059.

Wickham, J. C. 1995. Conventional insecticides, pp. 109–147. *In* M. K. Rust, J. M. Owens, and D. A. Reierson (eds.), Understanding and controlling the German cockroach. Oxford University Press, Oxford, United Kingdom.

Wu, X., and A. G. Appel. 2017. Insecticide resistance of several field-collected German cockroach (Dictyoptera: Blattellidae) strains. J. Econ. Entomol. 110: 1203–1209.