Investigation of Insecticide Resistance in Field-Collected German Cockroaches

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Introduction

The German cockroach, *Blattella germanica*, is one of the most prolific pests in the urban environment. This species is a source of allergens, pathogens, and psychological harm in indoor areas such as restaurants, apartments, schools, and other infrastructure. Control of the German cockroach has been largely dependent on synthetic insecticides. As a result of pesticide use and the rapid generation turnover of the cockroaches, insecticide resistance has developed and is a major obstacle in management of this species (Rust et al. 1995).

Detoxification by enzymes is a commonly documented mechanism of insecticide resistance in arthropod pests including the German cockroach. Known mechanisms include the activities of three enzyme families: esterase, cytochrome P450 monooxygenase (P450), and glutathione-S-tranferase (GST) (Yu 2014). The role of each enyzme may be unique to each cockroach strain. Understanding their contributions towards resistance of specific insecticides can allow for informed decision making for treatments.

We documented this information for several German cockroach field-collected strains. We used a topical contact method and bait evaluation method to profile resistance. Then, we tested any susceptibility changes towards deltamethrin and fipronil, two common insecticides, when combined with the insecticide synergists DEF, PBO, and DEM, which inhibit esterase, P450, and GST, respectively. Finally, enzymatic activity of the three families was measured for two field-collected strains to directly investigate their presence.

Methods

Cockroach Strains

Name	Collection Site	Type of Complex	Collection Date	Treatment History
UCR	-	-	-	Susceptible strain
WM	Los Angeles, CA	Public housing	2018	Various conventional
RG386	Los Angeles, CA	Public housing	2019	Various conventional
Ryan	San Jose, CA	Apartment	2020	Various conventional

Contact Toxicity of Insecticides Towards the Susceptible Strain. Adult males of the UCR strain were topically applied with ranges of doses of deltamethrin, fipronil, indoxacarb, abamectin, clothianidin, and hydramethylnon. Mortality was fitted to probit models to generate lethal dose (LD) values.

Diagnostic Dose Assessment. Adult males of field-collected strains WM, RG386, and Ryan were screened with the LD_{99} and 10 x LD_{99} of the UCR strain of all tested insecticides. Mortality of the field-collected strains was compared to the expected mortalities, 99% for LD_{99} and 100% for 10 x LD_{99}, of the UCR strain.

Bait Bioassay. Adult males of the UCR, WM, and RG386 strains were introduced into arenas with food, water, harborage, and 0.3 g of Maxforce FC Magnum (0.05% fipronil), Advion Evolution (0.6% indoxacarb), Maxforce Impact (1% clothianidin), Optigard (0.1% emamectin benzoate), or Siege gel bait (2% hydramethylnon). Mortality was fit to probit models to generate lethal time (LT) values.

Synergism. Adult males of WM, RG386, and Ryan were topically applied with DEF, PBO, or DEM, followed by applications of UCR's LD_{99} or 10 x LD_{99} of deltamethrin or fipronil. Mortality was compared to insecticide applications without synergists.

Measurement of Detoxification Enzymes. Adult males of UCR, WM, and RG386 were homogenized and enzymatic activity measured. Esterase activity was measured through 1-naphthyl acetate (1-NA), 2-naphthyl acetate (2-NA), and 4-nitrophenyl acetate (PNPA) assays. GST activity was measured through conjugation of chlorodinitrobenzene by GST. P450 titer was measured indirectly through detection of haem concentration.

Results

Table 1. Contact Toxicity of Insecticides Toward UCR Strain

Insecticide	n	LD_{50} (95% CI) (µg/insect)	LD_{99} (95% CI) (µg/insect)	Slope	(SE)	χ^2 (df)
deltamethrin	200	0.0056 (0.0037 - 0.0076)	0.025 (0.014 - 0.16)	3.555	0.447	2.3626 (2)
fipronil	270	0.00071 (0.00055 - 0.00084)	0.0048 (0.0034 - 0.0083)	2.81	0.396	3.235 (5)
indoxacarb	190	0.13 (0.09 - 0.16)	1.48 (0.86 - 4.33)	2.182	0.379	0.842 (3)
clothianidin	140	0.013 (0.011 - 0.018)	0.11 (0.055 - 0.47)	2.603	0.535	0.011 (2)
abamectin	120	0.0044 (0.0032 - 0.0053)	0.022 (0.014 - 0.064)	3.312	0.732	0.817 (2)
hydramethylnon	270	3.34 (1.70 - 5.18)	26.29 (13.17 - 191.37)	2.603	0.331	4.9970 (3)

Table 2. Diagnostic Dose Assessment

Insecticide	% Mortality from LD_{99}			% Mortality from 10 x LD_{99}		
	WM	RG386	Ryan	WM	RG386	Ryan
deltamethrin	0%*	0%*	0%*	10%*	15%*	5%*
fipronil	0%*	0%*	0%*	42%*	45%*	10%*
clothianidin	25%*	15%*	10%*	80%	90%	70%
indoxacarb	55%*	25%*	5%*	100%	85%	55%*
abamectin	55%*	75%*	85%	100%	100%	100%
hydramethylnon	75%*	45%*	30%*	-	-	-

*Indicates significant deviation from 99% mortality for LD_{99} and 100% mortality for 10 x LD_{99} based on Mantel-Haenszel tests.

Table 3. Bait Bioassay

Strain	Maxforce FC Magnum 0.05% fipronil	Maxforce Impact 1% clothianidin	Advion Evolution 0.6% indoxacarb	Optigard 0.1% emamectin benzoate	Siege 2% hydramethylnon
	LT_{50} (95% CI)[a]	LT_{50} (95% CI)[a]	LT_{50} (95% CI)[a]	LT_{50} (95% CI)[a]	LT_{50} (95% CI)[a]
UCR	12.5 (11.5 - 13.6)	11.6 (9.9 - 13.4)	8.8 (7.5 - 9.9)	28.4 (23.3 - 31.2)	45.5 (40.2 - 49.4)
WM	134.6 (108.5 - 174.7)	13.0 (10.3 - 16.1)	37.7 (33.6 - 42.2)	44.0 (38.7 - 49.7)	109.3 (99.7 - 119.6)
RG386	73.6 (64.9 - 83.8)	52.8 (33.4 - 80.5)	47.7 (38.2 - 56.6)	38.0 (31.2 - 44.9)	201.4 (178.2 - 225.2)

[a]Expressed in hours.

Table 4. Synergism

Insecticide	% Mortality of WM			% Mortality of RG386			% Mortality of Ryan		
	PBO	DEF	DEM	PBO	DEF	DEM	PBO	DEF	DEM
LD_{99} deltamethrin	35%*	10%	5%	65%*	10%	15%	50%*	30%*	0%
10 x LD_{99} deltamethrin	90%*	55%*	5%	100%*	60%*	45%	90%*	65%*	5%
LD_{99} fipronil	20%	10%	0%	0%	10%	10%	0%	5%	5%
10 x LD_{99} fipronil	90%*	85%*	45%	90%*	75%	50%	60%*	80%*	40%

*Indicates significant deviation from % mortality after insecticide treatment without synergist.

Table 5. Enzymatic Activity

Strain	Detoxification Enzymes				
	1-NA Esterase	2-NA Esterase	PNPA Esterase	P450	GST
UCR	91.88 ± 3.67	109.16 ± 3.39	519.10 ± 15.56	39.84 ± 2.12	0.37 ± 0.02
WM	118.6 ± 4.62*	142.60 ± 4.47*	633.60 ± 17.30	54.62 ± 3.67*	1.18 ± 0.06*
RG386	219.21 ± 11.60*	349.50 ± 19.93*	1494.31 ± 84.53 *	56.35 ± 2.80*	1.74 ± 0.14*

*Indicates significant difference from the UCR strain (Dunnett's test).

Discussion

All field-collected strains exhibited multiple insecticide resistances based on the diagnostic dose assessment (Table 2). In particular, they were highly resistant towards deltamethrin and fipronil, having significantly reduced mortality at both the LD_{99} and 10 x LD_{99} doses. Resistance was also detected towards abamectin, clothianidin, indoxacarb, and hydramethylnon at 1 x the LD_{99} but not at the 10 x LD_{99} level with a few exceptions. The high mortality observed with abamectin appears to translate over to the efficacy of Optigard (0.1% emamectin benzoate, same insecticidal class as abamectin) in the bait assay, with the 95% confidence intervals of the field-collected strain LT_{50}'s overlapping or marginally increased compared to the UCR strain (Table 3).

Combining the diagnostic doses of fipronil or deltamethrin with PBO or DEF significantly increased mortality for all field-collected strains, which suggests that resistance is caused by the activities of P450's and esterases (Table 4). This was further supported by direct analysis of enzymes which revealed elevated activity in all measured field-collected strains (Table 5). GST activity was also found to be elevated, but because the addition of DEM failed to influence mortality, GST's are unlikely to be main contributors of deltamethrin and fipronil resistance in these strains.

Conclusion

Although these data show a multitude of resistance problems in field-collected German cockroaches, they also reveal areas that can be exploited. Strategies utilizing the synergist PBO or products with abamectin, clothianidin, or indoxacarb may still be efficacious to control resistant German cockroaches in our current era. Future analysis of other field-collected strains will elucidate the effectiveness of these methods on a wider range of cockroach populations.

References

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