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Insecticide resistance and its potential mechanisms in field-collected German cockroaches (Blattodea: Ectobiidae) from Thailand

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We investigated insecticide resistance profiles of field populations of the German cockroach, *Blattella germanica* (L.), collected from central regions of Thailand. Seven strains (PW, RB, MTH, MTS, TL, AY, and SP) were evaluated with diagnostic doses (DD; 3 × LD_{95} generated from a susceptible strain) of deltamethrin, fipronil, and imidacloprid using topical assays and compared with a susceptible strain (DMSC). Results showed fipronil (2–27% mortality), deltamethrin (16–58% mortality), and imidacloprid (15–75% mortality) resistance in the field strains. Synergism studies with piperonyl butoxide (PBO) and *S,S,S*-tributyl phosphorotrithioate (DEF) in combination with the DD of insecticides significantly increased ($P < 0.05$) mortality of the test insects of the field strains suggesting the involvement of P450 monooxygenase and esterase pathways of detoxification. Gel bait evaluations demonstrated that all field-collected strains were resistant to Maxforce Forte (0.05% fipronil), Maxforce Fusion (2.15% imidacloprid), and Advion Cockroach Gel Bait (0.6% indoxacarb) with mean survival times ranging from 1.87–8.27, 1.77–11.72, and 1.19–3.56 days, respectively. Molecular detection revealed that the *Rdl* mutation was completely homozygous in all field-collected strains except in the PW strain. Field-collected strains were screened for 3 voltage-gated sodium channel (VGSC) mutations associated with pyrethroid resistance. The L993F mutation was present in 5 strains, but no C764R and E434K mutations were detected.

Key words: fipronil, deltamethrin, imidacloprid, *kdr*, *Rdl*

Introduction

The German cockroach, *Blattella germanica* (L.), is one of the most important indoor public health urban insect pests (Hu et al. 2020, Wang et al. 2021, Lee et al. 2022a). German cockroach infestations cause economic loss from contamination of foods and food preparation areas, medical costs from exposure to cockroach allergens, and costs associated with regulatory compliance (Schal and DeVries 2021, Wang et al. 2021). Chemical control remains the most reliable and effective method to control German cockroaches, and pest management professionals (PMPs) rely heavily on residual insecticide sprays and baits (Lee and Rust 2021).

Frequent usage of insecticides over many decades has led to the development of broad-scale insecticide resistance in the German cockroach, especially in recent years towards pyrethroids, phenylpyrazole (fipronil), neonicotinoids, and oxadiazine (indoxacarb) (Lee and Lee 2004, Chai and Lee 2010, Hu et al. 2020, 2021, Scharf and Gondhalekar 2021, González-Morales et al. 2022, Lee et al. 2022b). Resistance mechanisms identified in the German cockroach include metabolic resistance (cytochrome P450 monooxygenase, esterase, and glutathione S-transferase), reduced penetration, target-site insensitivity (*kdr* and *Rdl* mutations), and behavioral resistance (glucose aversion) (Siegfried and Scott 1991, Wu et al. 1998, Lee et al. 2000, 2022b, Valles et al. 2000, Chai and Lee 2010, Fardisi et al. 2019, Hu et al. 2021, Scharf and Gondhalekar 2021). Target-site insensitivity such as *kdr* mutations confer resistance to pyrethroids and DDT (Dong 1997, Dong et al.1998, Liu et al. 2000, Tan et al. 2002, Hu et al. 2021, Lee et al. 2022b), while the *Rdl* mutation confers resistance to fipronil

and cyclodienes (Ang et al. 2013, Scharf and Gondhalekar 2021, González-Morales et al. 2022).

In Southeast Asia, the German cockroach is a major insect pest in food preparation operations such as restaurant kitchens, food courts, and other commercial food handling facilities (Lee and Wang 2021). Similar to other parts of the world (Ko et al. 2016, Hu et al. 2020, Wang et al. 2021, Lee et al. 2022a), the control of the German cockroach in this region relies on using residual sprays and gel baits. Over the last 30 years, insecticide resistance in the German cockroach in Southeast Asia has been documented in Malaysia (Lee et al. 1996, Lee and Lee 2004), Singapore (Choo et al. 2000, Chai and Lee 2010), and Indonesia (Ahmad et al. 2009).

In Thailand, the control of German cockroaches in most commercial accounts is mainly carried out using residual sprays of pyrethroids (e.g., deltamethrin, cypermethrin, and bifenthrin), while gel baits are used when sprays are not permitted or when they are ineffective (Suchart Leelayouthyotin, Thailand Pest Management Association, personal communication). Even though there have been anecdotal reports of control failures on German cockroaches from PMPs in Thailand for many years, no information is available on the present status of insecticide resistance. Using DD, we measured resistance towards deltamethrin, fipronil, and imidacloprid in field-collected German cockroaches from 7 localities in central Thailand. Metabolic detoxification of deltamethrin, fipronil, and imidacloprid via P450 monooxygenase and esterase was evaluated with the synergists piperonyl butoxide (PBO) and *S,S,S*-tributyl phosphorotrithioate (DEF), respectively. The target-site mutations *Rdl* and *kdr* were screened with DNA sequencing for each strain. We also evaluated the performance of 3 commercial gel bait formulations containing fipronil, imidacloprid, and indoxacarb against these field-collected strains.

Materials and methods

Cockroach strains

Field populations of the German cockroach (PW, RB, MTH, MTS, TL, AY, and SP strains) were collected from different localities in central Thailand (Table 1) at random using glass jar traps provisioned with a piece of white bread moistened with 5 ml of beer as an attractant. They were brought back to the laboratory and reared in the Department of Entomology, Kasetsart University, Bangkok, for 2–3 generations to achieve enough numbers before they were used for the study. A susceptible strain (DMSC) originally obtained from the National Institute of Health, Ministry of Public Health, Thailand, was used for comparison. This strain has been reared for at least 3 years in the laboratory in Kasetsart University. All strains were reared under controlled environmental conditions of 25 ± 5 °C and 60 ± 10% relative humidity, with a 12:12 (L:D) photoperiod. The insects were provided dog chow (SmartHeart GOLD Mother & Baby Dog, Perfect Companion Group Co., Ltd., Bangkok, Thailand) and water ad libitum.

Table 1. Information on the German cockroach strains evaluated in this study

Strain	Site	City, Province	Collection date
DMSC	Lab susceptible		n/a
PW	Office building	Pathumwan, Bangkok	October 9, 2021
RB	Restaurant	Rattanathibet, Nonthaburi	November 23, 2021
MTH	Hotel kitchen	Muangthong Thani, Nonthaburi	January 25, 2022
MTS	Restaurant in shopping center	Muangthong Thani, Nonthaburi	December 28, 2021
TL	Restaurant kitchen	Thonglor, Bangkok	January 29, 2022
AY	Grocery shop	Phra Nakhon Si Ayutthaya	December 9, 2021
SP	Restaurant kitchen	Samut Prakan	February 15, 2022

Insecticides and synergists

Technical grade fipronil (95.37%) (Sherwood Corporation, Thailand), deltamethrin (99.62%) (LGC Ltd. Co., Thailand), imidacloprid (97.57%) (Sherwood Corporation, Thailand), piperonyl butoxide (PBO) (99%, Dr. Ehrenstorfer, LGC Ltd. Co., Thailand) and *S,S,S*-tributyl phosphorotrithioate (DEF) (100%, Chem Service Inc., West Chester, PA) diluted in acetone were used in this study. Gel baits containing 0.05% fipronil (Maxforce Forte, Bayer Cropscience, Thailand), 2.15% imidacloprid (Maxforce Fusion, Bayer Cropscience, Thailand), and 0.6% indoxacarb (Advion Cockroach Gel Bait, Syngenta Co., Ltd, Thailand) were used in the bait evaluation.

Topical bioassay

The procedures followed that of Lee et al. (2022b) with minor modifications. Adult males were used in all experiments because of their uniform sizes and physiological states, and removal of males from the culture has minimal impact on rearing (Lee et al. 2022a). Ten adult males of *B. germanica* were anesthetized with a light dose of CO_2 before being topically applied with a 0.5 µl droplet of a diagnostic dose (DD) of fipronil, deltamethrin, or imidacloprid on the abdominal sternites using a micropipette. The DD for fipronil was 3 × LD_{95} (11 ng/insect) of a susceptible strain reported earlier in Lee et al. (2022b). For deltamethrin, the DD used was 34 ng/insect (= 3 × LD_{95} in Lee et al. 2022b). The DD for imidacloprid was 21.22 µg/insect, which was ~3 × LD_{95} of the susceptible strain in Chai and Lee (2010) calculated from an average adult male body mass of ~52.6 mg. Mortality of the cockroaches was recorded at 10 min intervals up to 1 h and at 3, 6, 12, 24, 48, and 72 h. The controls were each treated with 0.5 µl acetone alone. All treated cockroaches were provided food and water *ad libitum*. Experiments were replicated 4–10 times for each insecticide.

Synergism

Two synergists were used to determine the possible involvement of cytochrome P450 monooxygenase and esterases: piperonyl butoxide (PBO) at 100 µg/insect and *S,S,S*-tributyl phosphorotrithioate (DEF) at 30 µg/insect, respectively. Ten adult males were anesthetized with CO_2, and 0.5 µl of the synergist was applied to the abdominal sternites with a micropipette. An hour after synergist application, the synergist-treated insects were treated with a DD of fipronil, deltamethrin, or imidacloprid. The treated cockroaches were provided with food and water ad libitum. Mortality of the cockroaches was recorded at 10 min intervals up to 1 h and at 3, 6, 12, 24, 48, and 72 h. Controls were treated with synergist + acetone. Each treatment was replicated 5 times.

Gel bait evaluations

Ten adult males were acclimatized for 48 h in a test arena (19 × 28 × 10 cm) with folded corrugated cardboard as harborage. The inner wall surface was lined with a thin layer of petroleum jelly (Vaseline, Unilever, Thailand) to prevent the cockroaches from escaping. Food (dog chow) and water were supplied ad libitum. A 0.3 g application of gel bait was introduced in a cut weigh boat at 1 corner of the test arena in the presence of food and water. The mortality of the cockroaches was observed every 2-h interval during the first 24 h and then every 12 h thereafter up to 14 days. Control sets received only food and water. The experiment for each strain was replicated 3 times.

Molecular detection of *kdr* and *Rdl* mutations

Adult males of each strain were kept in absolute ethanol and stored at 4 °C until extraction. The head and abdomen of each cockroach were removed before extraction. We followed the manufacturer's protocol of the DNeasy Blood and Tissue kit (Qiagen LLC, Germantown, MD) for the genomic DNA extraction from the thorax and legs. Extracted DNA was used immediately or stored at −20 °C before use. The amplification of the L993F, C764R, and E434K regions for *kdr* mutations and A302S for the *Rdl* mutation, the purification of PCR products, sequencing, and confirmation of mutations, followed the procedure described in Lee et al. (2022b) with minor modification. Ten males per strain were genotyped for both *kdr* and *Rdl* mutations (except for the PW strain for the *Rdl* mutation where 16 males were used).

Data analysis

Topical bioassay, synergism, and bait evaluation data were subjected to Kaplan–Meier survival analysis. Mantel–Cox log-rank tests (α = 0.05) were used to compare survivorship curves between each strain treated with insecticide alone, and insecticide + synergist. Spearman's correlation (α = 0.05) was calculated between the mean survival times obtained from the fipronil DD and that obtained from the evaluation using Maxforce Forte, and between the mean survival times from the imidacloprid DD and that obtained from the evaluation using Maxforce Fusion. All analyses were performed using SPSS Statistics version 25.0 (IBM Corporation, Armonk, NY).

Results

Topical bioassay and synergism

Diagnostic dose treatments on Thai field strains of the German cockroach revealed widespread fipronil, deltamethrin, and imidacloprid resistance (Tables 2–4, Fig. 1). Mortality of the field strains treated with fipronil ranged from 2 to 27% at 72 h, while mean survival times were 27.84 h for the DMSC susceptible strain versus 64.32–71.28 h for field strains (Fig 1A, Table 2). All field strains were resistant towards deltamethrin with increased survivorship and % mortality at 72 h ranging from 16 to 58% (Fig 1B, Table 3). Similarly, mortality from imidacloprid diagnostics ranged from 15 to 75% at 72 h with mean survival times of 28.69–63.66 h for field strains and 4.29 h for the DMSC susceptible strain (Table 4).

Pretreatment with the synergists PBO and DEF had a mixed impact on the toxicity of the fipronil diagnostic dose. The RB, MTH, TL,

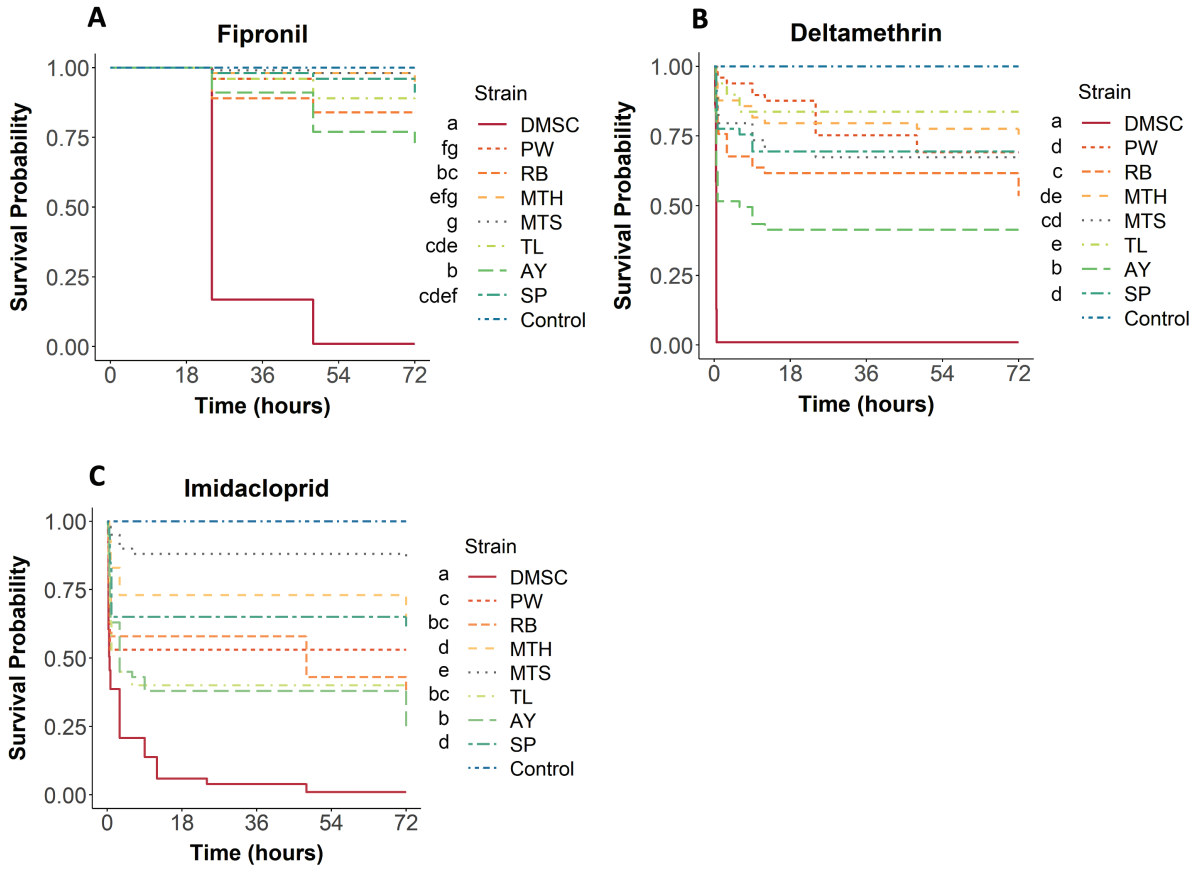

Fig. 1. Survivorship of cockroach strains exposed to A) fipronil, B) deltamethrin, and C) imidacloprid. Different lower-case letters indicate a significant difference in survivorship among strains (Log-rank test; α = 0.05).

Table 2. Mean survival time and % mortality at 72 h post-treatment of Thai field strains of the German cockroach after fipronil treatment alone (0.011 μg/insect) or in combination with synergists (PBO [100 μg/insect] and DEF [30 μg/insect])

Strain	Fipronil	Mean survival time (h)	95% CI	% mortality at 72 h
DMSC	Alone	27.84	26.11–29.57	100
	+ PBO	26.40	24.98–27.82	100
	+ DEF	26.88	25.34–28.42	100
PW	Alone	70.08	68.24–71.92	4
	+ PBO	70.08	68.11–72.05	8
	+ DEF	67.68	65.19–70.17	22
RB	Alone	65.52	62.48–68.56	16
	+ PBO	55.20	51.49–58.90	56
	+ DEF	57.12	53.99–60.25	74
MTH	Alone	71.04	69.57–72.51	5
	+ PBO	64.80	62.07–67.53	46
	+ DEF	62.88	59.89–65.87	46
MTS	Alone	71.28	70.24–72.32	2
	+ PBO	71.04	69.60–72.48	6
	+ DEF	70.08	68.24–71.92	4
TL	Alone	68.40	66.16–70.64	12
	+ PBO	62.40	59.55–65.25	46
	+ DEF	50.40	46.58–54.22	72
AY	Alone	64.32	61.30–67.34	27
	+ PBO	57.12	54.14–60.10	74
	+ DEF	61.44	58.41–64.46	58
SP	Alone	70.56	69.01–72.11	9
	+ PBO	71.04	69.66–72.42	12
	+ DEF	70.56	69.02–72.10	10

Table 3. Mean survival time and % mortality at 72 h post-treatment of Thai field strains of the German cockroach after deltamethrin treatment alone (0.034 μg/insect) or in combination with synergists (PBO [100 μg/insect] and DEF [30 μg/insect])

Strain	Deltamethrin	Mean survival time (h)	95% CI	% mortality at 72 h
DMSC	Alone	1.16	0.00–2.54	100
	+ PBO	0.96	0.00–2.35	100
	+ DEF	0.94	0.00–2.31	100
PW	Alone	56.38	51.42–61.35	30
	+ PBO	47.08	41.53–52.63	46
	+ DEF	34.79	28.64–40.93	62
RB	Alone	45.37	38.63–52.10	46
	+ PBO	24.84	18.44–31.25	76
	+ DEF	32.28	25.39–39.17	56
MTH	Alone	57.64	52.09–63.19	24
	+ PBO	45.47	39.04–51.90	42
	+ DEF	36.25	29.84–42.65	58
MTS	Alone	50.17	43.91–56.42	32
	+ PBO	39.15	32.43–45.87	48
	+ DEF	35.34	28.80–41.88	60
TL	Alone	60.77	55.73–65.81	16
	+ PBO	55.29	49.87–60.71	38
	+ DEF	41.37	35.09–47.66	56
AY	Alone	30.98	24.17–37.79	58
	+ PBO	13.18	88.52–17.83	90
	+ DEF	16.50	11.11–21.90	92
SP	Alone	50.77	44.42–57.11	30
	+ PBO	38.56	31.86–45.25	50
	+ DEF	37.56	30.90–44.21	68

and AY strains experienced a significant increase ($P < 0.05$) in total mortality with either synergist (~30–60%), while the PW, MTS, and SP strains were unaffected (Table 2). With deltamethrin, both PBO and DEF significantly ($P < 0.05$) increased the mortality of the test insects in all field strains at 72 h post-treatment (Table 3). Except for the PW strain pretreated with PBO, PBO, and DEF significantly decreased ($P < 0.05$) the survivorship of field strains treated with imidacloprid and caused up to ~60% increase in mortality at 72 h (Table 4).

Table 4. Mean survival time and % mortality at 72 h post-treatment of Thai field strains of the German cockroach after imidacloprid treatment alone (21.22 μg/insect) or in combination with synergists (PBO [100 μg/insect] and DEF [30 μg/insect])

Strain	Imidacloprid	Mean survival time (h)	95% CI	% mortality at 72 h
DMSC	Alone	4.29	2.52–6.07	100
	+ PBO	0.23	0.21–0.25	100
	+ DEF	0.22	0.19–0.24	100
PW	Alone	38.36	31.36–45.37	47
	+ PBO	27.76	20.98–34.55	62
	+ DEF	24.01	17.41–30.61	67
RB	Alone	38.41	31.85–44.96	62
	+ PBO	9.76	5.01–14.50	90
	+ DEF	11.10	6.09–16.11	85
MTH	Alone	52.99	46.78–59.20	37
	+ PBO	4.75	1.44–8.06	100
	+ DEF	11.02	6.00–16.04	85
MTS	Alone	63.66	59.08–68.25	15
	+ PBO	29.06	22.14–35.98	72
	+ DEF	36.14	29.11–43.17	50
TL	Alone	29.64	22.86–36.43	60
	+ PBO	6.12	2.31–9.93	92
	+ DEF	14.62	9.00–20.25	80
AY	Alone	28.69	21.98–35.39	75
	+ PBO	0.35	0.32–0.38	100
	+ DEF	6.05	2.24–9.86	92
SP	Alone	47.08	40.34–53.82	40
	+ PBO	16.87	10.92–22.81	80
	+ DEF	7.44	3.22–11.66	90

Gel bait evaluation

All field-collected strains were resistant towards Maxforce Forte and Maxforce Fusion baits. The DMSC susceptible strain tested with both baits had a mean survival time of <1 day, while field-collected strains ranged from 1.87 to 8.27 and 1.77 to 11.72 days for Maxforce Forte and Maxforce Fusion, respectively (Table 5, Fig. 2A and B). When tested with Maxforce Forte, MTH and MTS strains had the longest mean survival times (8.27 and 7.98 days, respectively), while RB and AY strains had mean survival times of <2 days (Table 5, Fig. 2A). Total % mortality at 14 days corresponded with mean survival time. There was correlation between the mean survival times of fipronil DD and those of Maxforce Forte ($\rho = 0.9286$, $P < 0.05$). For Maxforce Fusion, the TL strain had the longest mean survival time (>11 days) and the lowest % mortality at 14 days post-treatment (27%) (Table 5, Fig. 2B). AY strain showed the lowest mean survival time (<2 days). However, there was no correlation between the mean survival times of imidacloprid DD and those of Maxforce Fusion ($\rho = 0.2143$, $P > 0.05$).

With the exception of the RB strain, all field strains were resistant towards Advion based on significant differences ($P < 0.05$) in survivorship (Fig. 2C). None of the strains exceeded a 4 days mean survival time and % mortality at 14 days was ≥ 97% in all strains except TL, which was 87% (Table 5).

Target-site mutations

The *Rdl* mutation was completely homozygous in all field-collected strains except in the PW strain (Table 6), with 1 heterozygous and 5 susceptible individuals. No individuals with the *Rdl* mutation were

Table 5. Mean survival time and % mortality at 14 days post-treatment of Thai field strains of the German cockroach after commercial baits evaluations

Bait (% active ingredient)	Strain	Mean survival time (days)	95% CI	% mortality at 14 days
Maxforce Forte (Fipronil 0.05%)	DMSC	0.65	0.38–0.92	100
	PW	5.60	4.67–6.53	87
	RB	1.87	1.37–2.38	100
	MTH	8.27	7.42–9.13	73
	MTS	7.98	7.12–8.83	73
	TL	3.03	2.39–3.67	97
	AY	1.99	1.63–2.35	100
	SP	4.95	4.32–5.58	97
Maxforce Fusion (Imidacloprid 2.15%)	DMSC	0.59	0.31–0.86	100
	PW	3.54	2.86–4.22	100
	RB	5.22	4.27–6.17	80
	MTH	3.05	2.15–3.96	86
	MTS	2.36	1.97–2.75	100
	TL	11.72	10.80–12.63	27
	AY	1.77	1.39–2.15	100
	SP	2.97	2.28–3.66	93
Advion Cockroach Gel Bait (Indoxacarb 0.6%)	DMSC	0.85	0.58–1.11	100
	PW	1.94	1.57–2.32	100
	RB	1.19	0.90–1.49	100
	MTH	1.68	1.32–2.03	100
	MTS	2.23	1.89–2.58	100
	TL	3.56	2.74–4.38	87
	AY	1.67	1.21–2.14	97
	SP	2.47	1.92–3.03	97

found in the susceptible strain (DMSC). Three voltage-gated sodium channel mutations associated with pyrethroid resistance were screened. No C764R and E434K mutations were detected. Of the 7 field strains screened, 5 had the L993F mutation (Table 6). Mutation frequency was low in RB, MTH, and MTS strains which only had heterozygous or homozygous susceptible individuals. The TL strain had the highest frequency with 1 homozygous resistant, 8 heterozygous, and 1 susceptible individual. One individual from the AY strain possessed a G^{2927} to T substitution instead of G^{2927} to C typical of *kdr*, but this mutation still results in a phenylalanine substitution. The PW and SP strains did not have any individuals with *kdr* mutation.

Discussion

Deltamethrin resistance was present in all field strains and mortality ranged from 16 to 58% at 72 h post-treatment with the diagnostic dose, indicating a mixture of functionally susceptible and resistant individuals in the population (Fig. 1B, Table 3) (ffrench-Constant and Roush 1990, Robertson et al. 2017). This mixed resistance level is associated with a combination of metabolic detoxification and target-site insensitivity mechanisms. The pretreatment of piperonyl butoxide (PBO) and *S*,*S*,*S*-tributyl phosphorotrithioate (DEF) followed by deltamethrin significantly decreased ($P < 0.05$) survivorship compared to deltamethrin alone, which is observable in German cockroaches with P450 monooxygenase and esterase pathways of pyrethroid detoxification, respectively (Scott et al. 1990, Valles 1998, Chai and Lee 2010, Lee et al. 2022b) (Table 3). Further evidence of incomplete resistance selection is reflected in the low proportion of *kdr* mutations (Table 6). None of 7 strains screened were composed of a majority of L993F homozygous individuals, which is atypical of strains highly resistant towards pyrethroids (DeVries et al. 2019, Lee et al. 2022b). The TL and MTH strains had the highest mutation frequency at ~50%, while the PW and SP strains lacked *kdr* mutations altogether. The C764R and E434K mutations that confer high resistance levels were not detected in any strain (Tan et al. 2002). Although topical application and physiological resistance mechanism screening do not account for repellency, bioavailability or other behavioral effects that potentially reduce susceptibility in the field, the current data suggest that these strains have remained relatively physiologically susceptible to pyrethroids (Wu and Appel 2018).

Resistance towards fipronil was detected in all field strains considering the increased survivorships compared to the DMSC susceptible strain from treatment with the diagnostic dose of fipronil (Table 2, Fig. 1A). Like deltamethrin, the magnitude of resistance was heterogeneous and likely moderate based on the incomplete mortalities at 72 h post-treatment (Table 2). The *Rdl* mutation of the GABA-gated chloride channel was fixed in all sampled field strain individuals except in the PW strain, where 10 homozygous resistant, 1 heterozygous, and 5 susceptible individuals were found (Table 6). Although *Rdl* mutation was found at high frequency in all strains, it is not known to confer high level of fipronil resistance, as evident from previous studies (Kristensen et al. 2005, Ang et al. 2013, González-Morales et al. 2022). More variation was observed with metabolic detoxification. Although % mortality was low in the PW, MTS, and SP strains when treated with fipronil alone, the addition of synergists did not have a significant effect ($P > 0.05$). While synergism is potentially observable in these strains by using a higher dose, this data suggests a lack of detoxification at the 3 × LD_{95} level (Table 2). The remaining field strains, RB, MTH, TL, and AY, were affected by both PBO and DEF, which has been documented in other fipronil-resistant strains (Gondhalekar and Scharf 2012, Lee et al. 2022b). Fipronil does not undergo esterase-mediated hydrolysis due to the lack of ester linkages in its molecular structure, so the observed impact of DEF is likely due to P450 inhibition that may occur from high levels of the synergist (Scott 1990).

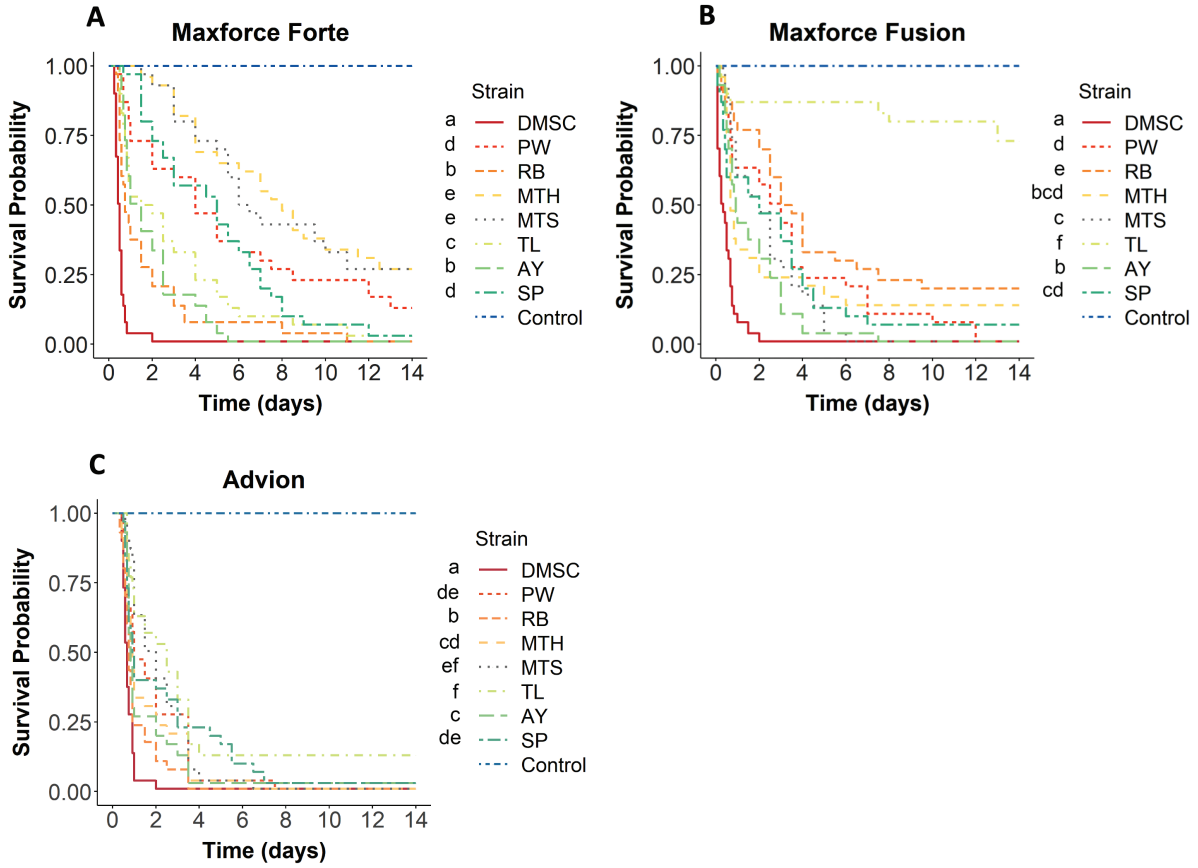

Fig. 2. Survivorship curves of Thai field cockroach strains evaluated against 3 commercial cockroach baits: A) Maxforce Forte, B) Maxforce Fusion, and C) Advion Cockroach Gel Bait. Different lower-case letters indicate a significant difference in survivorship among strains (Log-rank test; $\alpha = 0.05$).

Table 6. Prevalence of *kdr* and *Rdl* mutations in susceptible and field strains of the German cockroach collected in Thailand

	kdr (L993F)				*Rdl* (A302S)			
Strain	n	r/r	r/S	S/S	n	R/R	R/s	s/s
DMSC	10	0	0	10	10	0	0	10
PW	10	0	0	10	16	10	1	5
RB	10	0	3	7	10	10	0	0
MTH	10	0	5	5	10	10	0	0
MTS	10	0	2	8	10	10	0	0
TL	10	1	8	1	10	10	0	0
AY	10	1*	3	6	10	10	0	0
SP	10	0	0	10	10	10	0	0

*G^{2979} to T substitution.

While the diagnostic dose and *Rdl* screening results were similar between field strains, response towards Maxforce Forte (0.05% fipronil) varied significantly ($P < 0.05$) (Fig 2A, Table 5). Mean survival time ranged between 1.87 and 8.27 days, although all were significantly greater ($P < 0.05$) than the DMSC susceptible strain at 0.65 days. The treatment almost eliminated the RB, TL, AY, and SP strains with ≥ 97% mortality at 14 days despite prolonged survivorship. At the same time, a higher proportion of survivors was observed in the PW, MTH, and MTS strains (13–27%). This difference in resistance could be explained by factors not investigated in this study, such as extreme heterogeneity in resistance, contribution of bait aversion, or other mechanisms not identifiable by our approaches. The significant correlation ($P < 0.05$) between the fipronil topical diagnostic dose and Maxforce Forte survival times indicates that only physiological resistance was involved in affecting the susceptibility of these strains, ruling out the involvement of behavioral resistance.

Partial mortality from topically applied imidacloprid occurred across all field strains, showing varied levels of contact resistance (Fig. 1C, Table 4). Both PBO and DEF synergized imidacloprid, reducing survivorship and increasing overall mortality (from 15–75% to 50–100%), indicating a contribution of detoxification towards resistance (Table 4). The response to Maxforce Fusion (2.15% imidacloprid) contrasted between strains (Fig. 2B, Table 5). The RB, MTH, and SP strains ended at 80–93% total mortality, indicating a heterogeneous response, while the PW, MTS, and AY strains were eliminated. The TL strain, with a mean survival time of 11.72 days and 27% mortality at 14 days, was most resistant and is unlikely to be adequately controlled by this bait in the field. Low to moderate levels of resistance to imidacloprid are probably widespread in the German cockroach, but a strain at this level has seldom been reported (Chai and Lee 2010, Gondhalekar et al. 2011, Wu and Appel 2017). Interestingly, the TL strain was one of the least resistant field strains to topically applied imidacloprid based on average survival time (Fig. 1C, Table 4), suggesting a lack of physiological resistance in favor of altered behavioral response towards the formulation of Maxforce Fusion. More investigation is necessary to determine the mechanism of imidacloprid resistance in the TL strain, but this is an indication of a unique adaptation to prolonged exposure to the insecticide. The lack of correlation between the imidacloprid topical diagnostic dose and Maxforce Fusion survival times ($P > 0.05$) also

indicates that both physiological resistance and behavioral resistance (e.g., bait aversion), and possibly other factors were involved in the resistance of these strains.

Multiple assays showed the presence of resistance in all field strains in this study. The PW and MTS strains were incompletely killed by Maxforce Forte but reached complete mortality with Maxforce Fusion and Advion Cockroach Gel Bait (Table 5). The RB strain reached 100% mortality from Maxforce Forte and Advion Cockroach Gel Bait despite the subpar results of Maxforce Fusion (Table 5). The MTH and TL strains were nearly eliminated by Advion Cockroach Gel Bait and Maxforce Forte, respectively, even though they were resistant to other baits (Table 5). The AY and SP strains showed a lower level of resistance to all baits tested (Table 5).

However, although physiologically moderate, pyrethroid resistance is likely sufficient to cause unsatisfactory control when accounting for behavioral factors (Lee et al. 2022a). Overestimation of susceptibility from forced contact assays can occur due to the repellent properties of pyrethroid residuals at high concentrations. Management using compounds with marginal performance can be improved by supplementing with the combined or sequential use of more than one effective insecticide.

Caution should be exercised when inferring field efficacy from these experiments because only adult males were used. Females and nymphs can be less susceptible to insecticides due to lower foraging frequency (Metzger 1995). In addition, because multiple resistance mechanisms were already partially present, higher levels of resistance can be selected quickly from continuous exposure to the same insecticides if survivors remain after treatment (Fardisi et al. 2019). The current level of insecticide resistance in Thai German cockroach populations has the potential to result in the unsatisfactory performance of certain insecticides in the field. An emphasis on utilizing resistance intervention strategies such as insecticide rotations and mixtures to avoid the overuse of any single compound is necessary to maintain the cockroaches at a manageable level. Proper selection of effective insecticides is critical to ensure the continued control of German cockroaches in Thailand.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Rungarun Tisgratog (Conceptualization-Supporting, Data curation-Lead, Funding acquisition-Equal, Investigation-Lead, Project administration-Supporting, Resources-Equal, Validation-Equal, Visualization-Equal, Writing – original draft-Supporting, Writing – review & editing-Supporting), Chanikarn Panyafeang (Investigation-Supporting). Shao-Hung Lee (Formal analysis-Equal, Software-Supporting, Validation-Supporting, Visualization-Lead, Writing – original draft-Equal, Writing – review & editing-Supporting), Michael Rust (Methodology-Supporting, Resources-Supporting, Writing – original draft-Supporting, Writing – review & editing-Supporting), Chow-Yang Lee (Conceptualization-Lead, Formal analysis-Supporting, Funding acquisition-Equal, Methodology-Lead, Project administration-Lead, Resources-Lead, Software-Lead, Supervision-Lead, Writing – original draft-Equal, Writing – review & editing-Equal).

Supplementary Material

Supplementary data are available at the *Journal of Economic Entomology*.

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