



Household and Structural Insects

Characterization of insecticide resistance and their mechanisms in field populations of the German cockroach (*Blattodea: Ectobiidae*) in Taiwan under different treatment regimes

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This study investigated how management strategies influence resistance profiles in German cockroach (*Blattella germanica* (L.)) populations and their impact on the performance of commercial gel baits containing fipronil, imidacloprid, and indoxacarb. Field populations from premises managed under 3 different strategies: Baiting, random insecticide (RI) used, and insecticide rotation (IR) were tested. Almost all populations under RI and IR were resistant to deltamethrin, but low to moderate resistance was observed under the Baiting approach. Cytochrome P450 monooxygenases (P450) were involved in deltamethrin resistance in these resistant populations. All individuals under Baiting and RI were homozygous for the L993F mutation, but the populations under IR lacked homozygous-resistant individuals. Eighty-three percent of field populations with complete homozygosity for the *Rdl* mutation displayed low mortality upon exposure to 3x LD₉₅ fipronil. The effect of P450 and the *Rdl* mutation conferred high fipronil resistance in populations under the Baiting approach, recording moderate performance indices (PI) of 44–67 in fipronil bait. By contrast, those populations under RI and IR, in which involve glutathione S-transferases in fipronil resistance, had high PIs of 78–93. Almost 80% of populations exhibited over 90% mortality at 3x LD₉₅ indoxacarb treatment, accompanied by high PIs of 90–100 in indoxacarb bait. Partial mortality from 1x LD₉₅ imidacloprid occurred across all field populations due to the involvement of P450. PIs of imidacloprid bait ranged 5–57 and 20–94 in populations under RI and IR, respectively. Field populations demonstrate different resistance profiles depending on the treatment regimes, and the resistance mechanisms involved influenced gel bait's effectiveness.

Key words: metabolic resistance, target-site mutation, synergism, *kdr* resistance, topical bioassay

Introduction

Insecticide resistance poses an ongoing challenge in the control of German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae) infestations, with reports of resistance to 43 active ingredients documented to date (Mota-Sanchez and Wise 2024). Resistance mechanisms include metabolic resistance, target-site insensitivity, reduced penetration, and behavioral resistance (Scharf and Gondhalekar 2021).

Among the mechanisms, metabolism involving enhanced cytochrome P450 monooxygenase, esterase, and glutathione S-transferase activities is commonly involved in xenobiotic detoxification (Lee et al. 2000, Limoe et al. 2007). The involvement of

detoxification enzymes in mitigating the metabolic resistance in German cockroaches may vary across populations from the same region. For example, metabolism resistance involving enhanced cytochrome P450 monooxygenase majorly increased the tolerance of deltamethrin toxicity in 12 field populations of German cockroaches from Singapore (Chai and Lee 2010), while 8 populations had enhanced cytochrome P450 monooxygenase and esterases, conferring the deltamethrin resistance (Chai and Lee 2010). Limoe et al. (2007) reported that of 7 field populations of German cockroaches from Tehran, 2 populations had elevated levels of both cytochrome P450 monooxygenase and esterase; 4 populations had either increased cytochrome P450 monooxygenase or esterase

Table 1. Information on the susceptible and field-collected German cockroaches from premises under different treatment regimes used in this study

Group	Population	Mean weight of adult male \pm SE (g) ($n = 10$)	Treatment history/other information
Laboratory	EHI	0.0529 \pm 0.0010	Laboratory susceptible strain, no insecticide exposure
Baiting (baiting)	TC Da Restaurant	0.0525 \pm 0.0010	Pyrethroid residual spraying \rightarrow imidacloprid, dinotefuran, hydramethylnon containing baits
	TC Jo Restaurant	0.0601 \pm 0.0007	
	TC 2T Restaurant	0.0532 \pm 0.0017	
	TC Meat shop	0.0594 \pm 0.0016	
Random insecticide (RI)	TP Taipei	0.0556 \pm 0.0009	Residual spraying using pyrethroid, carbamate, imidacloprid and organophosphate, and fipronil, imidacloprid, dinotefuran, and hydramethylnon-containing baits randomly at each treatment
	TC Comestible	0.0523 \pm 0.0007	
	KS THSR	0.0535 \pm 0.0004	
	KS Zuoying mall	0.0522 \pm 0.0009	
Insecticide rotation (IR)	KS Pizza mall	0.0499 \pm 0.0016	Pyrethroids (cypermethrin, deltamethrin, cyphenothrin), neonicotinoid, and oxadiazine pesticides were rotated quarterly
	TY CPCS	0.0519 \pm 0.0007	
	TY EGSC	0.0538 \pm 0.0005	
	TP DTF Restaurant	0.0490 \pm 0.0021	

activities that responsible on permethrin resistance. The involvement of different detoxification enzymes in conferring permethrin and deltamethrin resistance also varied between populations from infested kitchens in Opelika, Alabama (Pridgeon et al. 2002). This may indicate that field populations demonstrate different metabolic-resistance profiles depending on their insecticide selection history or management types employed by pest management professionals (Scharf et al. 1997, 2022).

In addition to metabolic resistance, target-site insensitivity associated with mutations in the voltage-gated sodium channel gene, known as knockdown resistance (*knr*), has been shown to confer German cockroaches' resistance to pyrethroid insecticides (Chai and Lee 2010, Devries et al. 2019, Lee et al. 2022). For instance, *knr* mutation with lysine being substituted with phenylalanine amino acid (L993F) is linked to reduced susceptibility to pyrethroids (Dong 1997, Tan et al. 2002) and DDT (Scott and Matsumura 1981). Two other mutations, C764R and E434K, exacerbate reduced sodium channel sensitivity to deltamethrin (Tan et al. 2002). Individuals homozygous for the *knr* mutation exhibited a significantly higher resistance to pyrethroids than heterozygotes or wild-type cockroaches (Valles et al. 2003). Resistance to fipronil is conferred by alanine to serine substitution (A302S) on the resistance-to-dieldrin (*Rdl*) subunit of the γ -amino butyric acid (GABA)-gated chloride channel (Gondhalekar and Scharf 2012, Ang et al. 2013). German cockroaches with *Rdl* mutation, irrespective of the level of homozygosity, displayed moderate resistance to fipronil (Kristensen et al. 2005, Ang et al. 2013, Lee et al. 2022,). However, the combined effect of elevated cytochrome P450 monooxygenase and *Rdl* mutation substantially increases the fipronil resistance of German cockroaches (Gondhalekar and Scharf 2012).

A survey by Hu et al. (2020) on the insecticide resistance status of field German cockroach populations in Taiwan revealed high resistance to deltamethrin with resistance ratios ranging from 1.5 to 817.5 \times based on a time-course bioassay. Baiting is an effective control measure to suppress the German cockroach population. The first cockroach gel bait containing fipronil was commercially available in Taiwan in the 2000s. Since then, gel baits with other slow-acting active ingredients (i.e., imidacloprid, dinotefuran, hydramethylnon, and indoxacarb) have been gaining popularity. However, there were recent complaints by pest management professionals about their ineffectiveness of fipronil-containing gel bait.

In the present study, we investigated how management strategies influence resistance profiles in German cockroach populations and

subsequently impact the performance of commercial gel baits. To this end, cockroaches from premises that were serviced by 3 companies employing different strategies, namely baiting, random insecticide used, and insecticide resistance management, were collected. In the baiting approach, pyrethroid residual spraying was discontinued due to unsatisfactory population control. Thus, the residual spraying approach was replaced with baiting (Table 1). In the random insecticide used approach, residual sprayings using multiple active ingredients (AIs), including pyrethroids, neonicotinoids, organophosphate, and carbamate group insecticides, were randomly conducted every 2 wk. In the insecticide rotation management approach, residual sprayings using multiple AIs, including pyrethroids, neonicotinoids, and oxadiazine, were alternately employed every 4 mo.

Specifically, we examined the level of pyrethroid resistance and *knr* mutation frequency in the populations under different treatment regimes. It is hypothesized that pyrethroid resistance may be mitigated in the populations after pyrethroid residual spraying was discontinued under baiting compared to other approaches. We evaluated the susceptibility level of fipronil in German cockroaches and determined whether detoxifying enzymes or genetic adaptation confer their tolerance toward fipronil-containing commercial baits. We also determined the susceptibility of cockroach populations under random insecticide used and insecticide rotation approaches toward other non-pyrethroid active ingredients, i.e., indoxacarb and imidacloprid that were used for residual spraying relative to the baiting approach, their possible resistance mechanisms involved and the performance of imidacloprid- and indoxacarb-containing commercial baits.

Materials and Methods

Cockroach Collection and Rearing

Blattella germanica was collected from 12 premises in Taiwan between 2017 and 2021. The German cockroach infestation in the premises was serviced by 3 respective pest control companies that used different management approaches, namely baiting (imidacloprid, dinotefuran, hydramethylnon containing baits) after failure of pyrethroid spraying (hereby referred as Baiting), random insecticides (random use of pyrethroid, carbamate, imidacloprid, organophosphate, and fipronil, imidacloprid, dinotefuran, hydramethylnon containing baits, hereby referred as RI), insecticide rotation (quarterly rotation of pyrethroid (cypermethrin, deltamethrin, cyphenothrin), neonicotinoid, and oxadiazine, hereby referred as IR). Generally, the

premises were treated every 2 wk (Table 1). However, information on how long the treatment programs were conducted is unavailable. A susceptible laboratory strain (EHI) that has been reared in the laboratory without any insecticide exposure for more than 50 yr, was used for comparison. All German cockroaches were maintained under laboratory conditions of $25 \pm 1^\circ\text{C}$, $50 \pm 5\%$ RH, and a photoperiod of 12:12 h light:dark in round polyethylene containers (24 cm diameter \times 32 cm height) provided with corrugated cardboard harborage, dog food (RT-Mart beef flavor dog food, Hsinchu, Taiwan), and water ad libitum. The cockroaches were mass-reared under laboratory conditions for 6–12 mo. Before the experiment, adult male cockroaches were collected and transferred into a polyethylene cup, provisioned with water in a cotton ball, and starved for 24 h.

Insecticides

In this study, we used technical grade deltamethrin (98%, Tagros Chemicals India Ltd., Chennai, India), fipronil (98%, Toronto Research Chemicals, Inc., North York, Canada), indoxacarb (90%, Shangdong Jingbo Agrochemicals Technology Co. Ltd., Shangdong, China), and imidacloprid (94%, Tagros Chemicals India Ltd., Chennai, India). A stock solution of these chemicals was prepared by dissolving the insecticide in acetone (Union Chemical Works Ltd., Hsinchu, Taiwan). Commercial baits tested using Ebeling choice box were Jin-Li-Hai Ultra Max cockroach gel bait containing 0.05% fipronil (Kukbo Science Co., Ltd, Cheongju-si, Korea), Premise cockroach gel bait containing 2.15% imidacloprid (Bayer AG, Petaling Jaya, Malaysia), and Ke Mie Zhang cockroach gel bait containing 0.6% indoxacarb (ChungHsi Chemical Plant, Ltd, Hsinchu, Taiwan).

Topical Bioassays

Adult male cockroaches were collected and transferred into a polyethylene cup, provisioned with water in a cotton ball, and starved for 24 h before topical bioassays.

The test dosage of each insecticide is based on the LD_{95} of the respective insecticides tested on laboratory susceptible strain (EHI) as described in [Chai and Lee \(2010\)](#). Diagnostic doses of $1 \times \text{LD}_{95}$, $3 \times \text{LD}_{95}$, and $10 \times \text{LD}_{95}$ were used to assess the intensity of insecticide resistance based on the percentage of mortality (Table 2). Ten adult males of each cockroach population were anesthetized with CO_2 , and 1 μl of each insecticide was applied onto the first abdominal sternite using a micro-applicator (Burkard Scientific Ltd., Middlesex, UK). For control, the male cockroaches were treated with acetone only. Treated cockroaches were provided with water and dog food. The mortality was recorded at 72 h post-treatment. Each insecticide and dose was replicated 3 times.

Synergism of Insecticides

Combining insecticides with enzyme inhibitors was employed to determine the enzymes involved in resistance mechanisms. Synergism tests were conducted using piperonyl butoxide (PBO) (90%, Sigma-Aldrich Corporation, St. Louis, MO), S,S,S -tributyl

phosphorotrithioate (DEF) (100%, Chem Service Inc., West Chester, PA), and diethyl maleate (DEM) (97% Sigma-Aldrich Corporation, St. Louis, MO) to determine the involvement of cytochrome P450 monooxygenases, esterases, and glutathione S-transferases, respectively. Each synergist was diluted separately in acetone. Ten adult male cockroaches were anesthetized with CO_2 . One microliter of PBO, DEF, or DEM was applied at a rate of 100, 30, and 100 μg per cockroach, respectively, onto the first abdominal sternites. Then, insecticide was applied to synergist-treated German cockroaches using the same procedure. Treated cockroaches were provided food and water. The total mortality of cockroaches from deltamethrin treatment was recorded at 48 h, while fipronil, indoxacarb, and imidacloprid mortality was recorded at 72 h due to their slow-acting insecticidal effect. Controls were treated with acetone, PBO, DEF, and DEM only. The tests were replicated 3 times.

Detection of Target-site Mutations

Ten adult males from each population, including the EHI strain, were used to detect the presence of target-site mutations. Genomic DNA was extracted from individuals without heads and wings following the manufacturer's protocol from the GeneAll Exgene Tissue SV Plus mini kit (Geneall Biotechnology Co., Ltd, Korea). The amplification of the L993F, C764R, and E434K regions for *kdr* mutations and A302S for the *Rdl* mutation was performed using site-specific primers (Supplementary Table 1) as described in [Lee et al. \(2022\)](#). The PCR reactions were conducted in 50 μl total volumes with 25 μl of AppTaq PCR MasterMix (2 \times) (GeneTeks BioScience, Inc., Taiwan), 2 μl of each of forward and reverse primers (10 μM), 2 μl of template DNA (100–200 ng), and 21 μl of nuclease-free water. The PCR conditions used for amplification of L993F with PCR parameters of 95°C for 5 min, 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 10 min. The C764R region was amplified with PCR parameters of 95°C for 3 min, 40 cycles of 94°C for 45 s, 55°C for 45 s, 72°C for 45 s, and a final extension at 72°C for 5 min ([Devries et al. 2019](#)). The E434K region was amplified with 95°C for 5 min, a 10-step touchdown cycle of 95°C for 15 s, 60 – 55°C (decreasing by increments of 0.5°C) for 15 s, and 72°C for 15 s followed by 35 cycles of 95°C for 15 s, 50°C for 15 s, 72°C for 15 s, and a final extension of 72°C for 10 min. The PCR parameters for A302S followed that of [Lee et al. \(2022\)](#) with a slight modification: 94°C for 5 min, 40 cycles of 94°C for 30 s, 62.5°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 10 min. The PCR product was used to verify proper-sized bands on 2% agarose gel electrophoresis. The amplicons were purified and sequenced by the Tri-I Biotech (Taipei, Taiwan). The sequence chromatograms were utilized to determine the genotypes of the mutations.

Ebeling Choice Box Tests

The toxicity, repellency, and potential performance of the bait formulations were determined using Ebeling choice boxes ([Ebeling et al. 1966](#), [Zurek et al. 2003](#), [Appel et al. 2022](#), [Lee et al. 2022](#)). The choice box was a square wooden box (33 \times 33 cm) with 2 equally

Table 2. Information on LD_{95} values was obtained from [Chai and Lee \(2010\)](#)

Insecticide	$1 \times \text{LD}_{95}$ ($\mu\text{g}/\text{g}$)	$3 \times \text{LD}_{95}$ ($\mu\text{g}/\text{g}$)	$10 \times \text{LD}_{95}$ ($\mu\text{g}/\text{g}$)
Deltamethrin	0.6	1.8	6
Fipronil	0.1	0.3	1
Indoxacarb	60	180	600
Imidacloprid	140	420	1400

sized compartments (33 × 16.5 cm) divided by a wooden wall. A hole in the top center of the dividing wall allows the cockroaches to move freely between compartments. Both compartments were covered with a piece of plexiglass. Additionally, one compartment was covered with a piece of cardboard to create a dark environment. The light compartment consists of dog food and water, while the dark compartment consists of 0.5 g gel bait on a petri dish (diameter: 0.55 × 0.15 cm). For control, the dark compartment contains no gel bait. Twenty adult male cockroaches of each population were collected into plastic cups and starved for 24 h before being introduced into a light compartment. The choice boxes were placed in a laboratory condition on a photoperiod of 12:12 h (L:D). The number of dead and live cockroaches in the light and dark compartments was recorded daily for 7 days. The experiments were replicated 3 times.

Data Analysis

The percentage of mortality was subjected to arcsine square root transformation before analysis. For the significance of resistance of field-collected populations, the differences in the percentage of mortality among field populations exposed to doses of 1× LD₉₅, 3× LD₉₅, and 10× LD₉₅ of test insecticides were compared using ANOVA, and the means were separated using post hoc Tukey's HSD. Additionally, the mortalities resulting from synergist + insecticide were compared with those respective treatments with insecticide alone using Student's *t*-test. All analyses in this study were conducted in SPSS analysis version 11.0 (SPSS Inc., Chicago, IL) at $\alpha = 0.05$. For the choice box, the performance index (PI) was calculated. The PI evaluates the effects of mortality and repellency of a test product and estimates its potential field performance. The PI is calculated as follows:

$$PI = 1 - \left(\frac{\text{Number alive} + \text{Number alive in light side}}{\text{Number dead} + \text{Initial total number}} \right) \times 100$$

PI values of -100, +100, and 0 indicate complete repellency and no mortality, complete mortality and no repellency, and no repellency and no mortality, respectively (Appel et al. 2022, Lee et al. 2022). The differences in PI of a commercial gel baits among field populations were compared using ANOVA and the means of PI were separated using post hoc Tukey's HSD.

Results

Topical Bioassays

Almost no mortality was observed for each population in control treatments (Supplementary Table 2). At the highest dose of deltamethrin (10× LD₉₅), significant limited mortality (<40%) was observed in 3 out of 5 and 3 out of 3 field populations under RI and IR ($F = 71.5$, $df = 12, 26$, $P < 0.001$), which using pyrethroid residual spraying alternately, respectively. In contrast, populations managed under Baiting, in which the application of pyrethroid residual spraying was withdrawn, experienced over 90% mortality when exposed to the same deltamethrin dose (Table 3).

Fipronil induced almost no mortality in 10 of 12 field populations at doses of 3× and 1× LD₉₅. At the highest dose (10× LD₉₅), 8 of 12 field populations had less than 40% mortality (Table 4).

Five populations under RI and IR recorded significantly low mortality (mortality ranged from 37% to 67%) when exposed to indoxacarb at the lowest dose among populations tested ($F = 102.8$, $df = 12, 26$, $P < 0.001$). The mortality of these resistant populations increased to more than 70% upon exposure to 3× LD₉₅ of indoxacarb (Table 5).

For imidacloprid, 11 out of 12 populations showed significantly less mortality, ranging from 0% to 77% at 1× LD₉₅ imidacloprid ($F = 86.03$, $df = 12, 26$, $P < 0.001$) compared to EHI (Table 6). However, when treated with 10× LD₉₅ imidacloprid, 9 out of 12 populations had 90%–100% mortality irrespective of treatment regimes.

Synergism of Insecticides

In synergism tests, among the 3 populations under RI with less than 40% mortality at 10× LD₉₅ deltamethrin, PBO significantly increased mortality in KS Zuoying from 20% to 60% but did not significantly affect mortality in the other 2 populations (KS THSR and KS Pizza Mall). Similarly, of the 3 populations under IR, mortality in TC CPCS and TY EGSC increased significantly from 17% to 55% and 3% to 55%, respectively, with PBO pretreatment. However, although significant, PBO pretreatment only partially negated deltamethrin resistance in these populations (KS Zuoying, TC CPCS, TY EGSC). DEM and DEF synergists had minimal impact on mortality at all doses of deltamethrin except for TC Da Restaurant, which experienced a moderate increment in mortality after exposure to 1× LD₉₅ deltamethrin (Table 3).

The effect of synergists on the toxicity of fipronil was more pronounced at the highest dose tested. PBO increased the toxicity of fipronil for TC Da Restaurant and TC Jo Restaurant under the Baiting approach from 0% to 70%–90%. At the same time, DEF and DEM had no impact on the mortality of these 2 populations. Nevertheless, the pretreatment of DEF and DEM significantly increased the toxicity of fipronil in most populations under RI and IR, increasing the mortality from 7%–63% to 65%–100% (Table 4).

In the indoxacarb bioassay, the pretreatment of DEM and DEF significantly increased the mortality of TC Comestible under RI from 43% to 85%–100%; however, their effects were antagonistic, as evidenced by a significant decrease in mortalities in most populations (Table 5). Overall, the pretreatment of PBO had a negligible effect on indoxacarb toxicity.

PBO synergism on imidacloprid significantly increased susceptibility in most populations at a dose of 1× LD₉₅, regardless of the types of management strategies, with mortality increased from 10%–77% to 80%–100%. However, the KS Pizza Mall population under RI required a higher dose (10× LD₉₅) with PBO synergists to achieve 95% mortality. The effects of DEM and DEF synergists were mixed, with a significant increase in mortality observed in some populations, including TC Jo Restaurant, TC Da Restaurant, TC 2T Restaurant under the Baiting approach, TY EGSC under IR, TC comestible under RI (Table 6).

Detection of Target-site Mutations

In the 4 field populations under the Baiting approach, the L993F *kdr* mutation was present at a high frequency in 2 field populations, with 10%–30% of individuals characterized with homozygous resistant genotypes and 50%–70% of individuals with heterozygous genotypes. Under RI management, 3 out of 5 populations had a high mutation frequency, with 10%–40% of homozygous resistant individuals and 40%–60% of heterozygous resistant individuals. All field populations under IR had a low mutation frequency, with only 30%–60% heterozygous and 40%–70% susceptible individuals and no homozygous resistant individuals. No C764R and E434K mutations were found in all populations tested.

Among the 12 field populations surveyed, the A302S *Rdl* mutation was present in 10 populations at a high frequency (100%) although fipronil is not used in current Baiting and IR approaches.

Table 3. Percentage mortality of field collected German cockroaches after treatment with the diagnostic doses of deltamethrin (De) and synergists

Strategy	Population	Mean % mortality ± SEM											
		1x				3x				10x			
		De only	De + PBO	De + DEM	De + DEF	De only	De + PBO	De + DEM	De + DEF	De only	De + PBO	De + DEM	De + DEF
Baiting	EHI	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	TC Da Restaurant	10.0 ± 0.0b	50.0 ± 0.4*	75.0 ± 0.2*	35.0 ± 0.2*	73.3 ± 1.0a	100.0 ± 0.0	80.0 ± 0.0	75.0 ± 0.2	96.7 ± 0.2a	100.0 ± 0.0	95.0 ± 0.2	90.0 ± 0.0
	TC Jo Restaurant	50.0 ± 0.0c	50.0 ± 0.0	0.0 ± 0.0*	35.0 ± 0.2	83.3 ± 0.5a	100.0 ± 0.0	15.0 ± 0.2*	65.0 ± 0.2	93.3 ± 0.2a	100.0 ± 0.0	60.0 ± 0.9	85.0 ± 0.2
	TC 2T Restaurant	93.3 ± 0.2a	100.0 ± 0.0	80.0 ± 0.4	85.0 ± 0.2	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Random insecticide (RI)	TC Meat shop	80.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.2	83.3 ± 0.5a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.3 ± 0.2a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	TP Taipei	6.7 ± 0.2b	15.0 ± 0.2	5.0 ± 0.2	0.0 ± 0.0	26.7 ± 0.7bc	75.0 ± 0.2	40.0 ± 0.4	25.0 ± 0.2	83.3 ± 0.5a	90.0 ± 0.0	55.0 ± 0.2	25.0 ± 0.2
	TC Comestible	26.7 ± 0.4d	95.0 ± 0.2*	10.0 ± 0.4	0.0 ± 0.0	63.3 ± 0.2ac	100.0 ± 0.0	50.0 ± 0.0	55.0 ± 0.2	90.0 ± 0.3a	100.0 ± 0.0	65.0 ± 0.2	75.0 ± 0.2
	KS THSR	0.0 ± 0.0b	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0b	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0b	5.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Insecticide rotation (IR)	KS Zuoying mall	10.0 ± 0.3b	10.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 0.5b	20.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	20.0 ± 0.3b	60.0 ± 0.0*	5.0 ± 0.2	0.0 ± 0.0
	KS Pizza mall	13.3 ± 0.2b	15.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	16.7 ± 0.5b	25.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	36.7 ± 0.5c	40.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	TY CPCS	0.0 ± 0.0b	50.0 ± 0.0*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0b	50.0 ± 0.0*	10.0 ± 0.0	0.0 ± 0.0	16.7 ± 0.5b	55.0 ± 0.2*	15.0 ± 0.2	0.0 ± 0.0
	TY EGSC	0.0 ± 0.0b	5.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 0.2b	5.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0b	5.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
TP DTF Restaurant	3.3 ± 0.2b	15.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 0.2b	55.0 ± 0.2*	5.0 ± 0.2	0.0 ± 0.0	3.3 ± 0.2b	55.0 ± 0.2*	5.0 ± 0.2	0.0 ± 0.0	

Mean mortality followed by the different letters within the same doses in deltamethrin treatment only significantly different (Tukey's HSD; $P < 0.05$). Mean mortality with an asterisk within the same row of the same population indicates that it is significantly different from that of deltamethrin treatment only ($P < 0.05$; t -test).

TC Meat Shop had 30% homozygous resistant, 60% heterozygous resistant, and 10% susceptible individuals, and TC 2T Restaurant had 30% heterozygous resistant and 70% susceptible individuals under the Baiting approach (Table 7). The relatively low A302S *Rdl* mutation frequency in the TC Meat Shop and TC 2T Restaurant was supported by their susceptibility to fipronil, with 80%–97% mortality recorded at a dose of $3 \times LD_{95}$ fipronil.

Ebeling Choice Box Tests

No to limited mortality was observed in control. All cockroaches were observed to hide in the dark chamber (Supplementary Table 3). The PI for EHI were consistently 100, indicating complete mortality and no repellency in all tested commercial products. The results revealed that out of 4 populations under the Baiting approach, 2 populations, namely, TC 2T Restaurant and TC Meat Shop that showed the absence of *Rdl* mutation and metabolic resistance recorded higher PIs of >95 on day 1 (Fig. 1) after feeding on fipronil-containing bait compared to TC Da Restaurant and TC Jo Restaurant (PIs of <15) ($F = 349.6$, $df = 3, 8$, $P < 0.001$). It is worth noting that *Rdl* mutation and cytochrome P450 are majorly involved in increasing fipronil resistance in TC Da Restaurant and TC Jo Restaurant populations. The result is consistent with the TP Taipei population under RI (PI = 20, $F = 19.39$, $df = 4, 10$, $P < 0.001$) and TP DTF Restaurant under IR (PI = 67, $F = 3.086$, $df = 2, 6$, $P < 0.05$) had significantly lower PIs at day 7 compared to other populations within the treatment regimes (Fig. 1). In contrast, populations under RI (TC Comestible, KS THSR, KS Zuoying mall) and IR (TY CPCS, TY EGSC) that had combined metabolic resistance involving hydrolytic and GST activities and *Rdl* mutation had PIs of >80 (Fig. 1).

Overall, indoxacarb caused a PI value of more than 80 by day 7 post-treatment in most populations tested, which was significantly higher than those of TY CPCS and TP DTF Restaurant under IR ($F = 21.96$, $df = 11, 24$, $P < 0.001$). However, the PI values recorded in TY CPCS and TP DTF Restaurant populations still remained above 70 (Fig. 1).

In the evaluation of imidacloprid-containing bait, all field populations under the Baiting approach had significantly higher PIs of >97 compared to those of populations under RI and IR (PIs ranged from 5 to 57) ($F = 26.83$, $df = 2, 33$, $P < 0.001$). Notably, residual spraying with pyrethroids was applied in the premises under RI and IR. In addition, the KS Pizza Mall and TP Taipei populations showed the lowest PIs, with mortality of less than 5% (Fig. 1).

Discussion

The present study demonstrated that the treatment regimes employed by pest management professionals shape insecticide resistance profiles and the mechanisms involved in pyrethroid resistance in field populations. Fipronil resistance and *Rdl* mutation remained high in populations irrespective of treatment regimes. However, the metabolic mechanisms resulting from treatment regimes determine the PI of fipronil- and imidacloprid-containing bait. The effect of treatment regimes on susceptibility toward indoxacarb was negligible.

Laboratory assays revealed that the diagnostic dose at $3 \times LD_{95}$ deltamethrin caused high mortality in field-collected populations under the Baiting approach, with over 70% mortality recorded. In contrast, German cockroach populations collected from premises under RI and IR exhibited resistance to deltamethrin. Almost all treated cockroaches survived the diagnostic dose at $3 \times LD_{95}$ and mortality did not exceed 40% when exposed to the diagnostic dose

Table 4. Percentage mortality of field collected German cockroaches after treatment with the diagnostic doses of fipronil (Fi) and synergists

Strategy	Population	Mean % mortality \pm SEM											
		1x				3x				10x			
		Fi only	Fi + PBO	Fi + DEM	Fi + DEF	Fi only	Fi + PBO	Fi + DEM	Fi + DEF	Fi only	Fi + PBO	Fi + DEM	Fi + DEF
Baiting	EHI	100.0 \pm 0.0a	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0a	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0a	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0
	TC Da Res- taurant	0.0 \pm 0.0b	20.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0b	85.0 \pm 0.2*	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0b	90.0 \pm 0.0*	0.0 \pm 0.0	0.0 \pm 0.0
	TC Jo Res- taurant	0.0 \pm 0.0b	5.0 \pm 0.2	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0b	10.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0b	70.0 \pm 0.0*	0.0 \pm 0.0	0.0 \pm 0.0
	TC 2T Res- taurant	0.0 \pm 0.0b	85.0 \pm 0.2*	25 \pm 0.2*	0.0 \pm 0.0	80.0 \pm 0.0a	95.0 \pm 0.2	75.0 \pm 0.2	0.0 \pm 0.0*	96.7 \pm 0.2a	100.0 \pm 0.0	100.0 \pm 0.0	70.0 \pm 0.0*
Random insecticide (RI)	TC Meat shop	56.7 \pm 0.4c	100.0 \pm 0.0*	65.0 \pm 0.2	75.0 \pm 0.2	96.7 \pm 0.2a	100.0 \pm 0.0	100.0 \pm 0.0	80.0 \pm 0.0	96.7 \pm 0.2a	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0
	TP Taipei	0.0 \pm 0.0b	5.0 \pm 0.2	10.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0b	10.0 \pm 0.0	15.0 \pm 0.2	0.0 \pm 0.0	0.0 \pm 0.0b	65.0 \pm 0.2*	15.0 \pm 0.2	5.0 \pm 0.2
	TC Comes- table	0.0 \pm 0.0b	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0b	45.0 \pm 0.2*	0.0 \pm 0.0	5.0 \pm 0.2	23.3 \pm 0.2b	55.0 \pm 0.2*	100.0 \pm 0.0*	100.0 \pm 0.0*
	KS THSR	0.0 \pm 0.0b	0.0 \pm 0.0	25.0 \pm 0.2*	0.0 \pm 0.0	0.0 \pm 0.0b	0.0 \pm 0.0	65.0 \pm 0.2*	0.0 \pm 0.0	6.7 \pm 0.2b	10.0 \pm 0.0	75.0 \pm 0.2*	100.0 \pm 0.0*
Insecticide rotation (IR)	KS Zuoying mall	0.0 \pm 0.0b	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0b	15.0 \pm 0.2	10.0 \pm 0.0	5.0 \pm 0.2	73.3 \pm 0.2c	70.0 \pm 0.0	100.0 \pm 0.0*	95.0 \pm 0.2*
	KS Pizza mall	0.0 \pm 0.0b	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	6.7 \pm 0.2b	0.0 \pm 0.0	5.0 \pm 0.2	5.0 \pm 0.2	20.0 \pm 0.5b	0.0 \pm 0.0	25.0 \pm 0.7	80.0 \pm 0.0*
	TY CPCS	0.0 \pm 0.0b	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0b	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	26.7 \pm 0.2b	25.0 \pm 0.2	75.0 \pm 0.2*	65.0 \pm 0.2*
	TP DTF Res- taurant	0.0 \pm 0.0b	5.0 \pm 0.2	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0b	10.0 \pm 0.2	5.0 \pm 0.2	10.0 \pm 0.0	63.3 \pm 0.4c	60.0 \pm 0.0	95.0 \pm 0.2*	100.0 \pm 0.0*

Mean mortality followed by the different letters within the same doses in deltamethrin treatment only significantly different (Tukey's HSD; $P < 0.05$).

Mean mortality within the same row of the same population indicates that it is significantly different from that of fipronil treatment only (Student's t -test; $P < 0.05$).

Table 5. Percentage mortality of field collected German cockroaches after treatment with the diagnostic dose of indoxacarb (In) and synergists

Strategy	Population	Mean % mortality ± SEM											
		1x				3x				10x			
		In only	In + PBO	In + DEM	In + DEF	In only	In + PBO	In + DEM	In + DEF	In only	In + PBO	In + DEM	In + DEF
Baiting	EHI	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	TC Da Res- taurant	100.0 ± 0.0a	100.0 ± 0.0	50.0 ± 0.4*	35.0 ± 0.2*	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	70.0 ± 0.0*	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	TC Jo Res- taurant	93.3 ± 0.0a	95.0 ± 0.2	95.0 ± 0.0	75.0 ± 0.2*	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	95.0 ± 0.2	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Random insecticide (RI)	TC 2T Res- taurant	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	TC Meat shop	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	TP Taipei	100.0 ± 0.0a	100.0 ± 0.0	35.0 ± 0.2*	35.0 ± 0.2*	100.0 ± 0.0a	100.0 ± 0.0	90.0 ± 0.2	95.0 ± 0.2	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Insecticide rotation (IR)	TC Comes- tible	43.3 ± 0.2b	45.0 ± 0.2	100.0 ± 0.0*	85.0 ± 0.2*	96.7 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	KS THSR	63.3 ± 0.5b	70.0 ± 0.0	100.0 ± 0.0	70.0 ± 0.4	73.3 ± 0.5b	85.0 ± 0.2	100.0 ± 0.0	90.0 ± 0.0	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	KS Zuoying mall	100.0 ± 0.0a	100.0 ± 0.0	45.0 ± 0.2*	80.0 ± 0.0	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Insecticide rotation (IR)	KS Pizza mall	36.7 ± 0.5b	60.0 ± 0.4	20.0 ± 0.0*	75.0 ± 0.2	76.7 ± 0.5b	85.0 ± 0.2	40.0 ± 0.4	95.0 ± 0.2	96.7 ± 0.2a	95.0 ± 0.2	85.0 ± 0.2	100.0 ± 0.0
	TY CPCS	66.7 ± 0.2b	75.0 ± 0.2	35.0 ± 0.2*	30.0 ± 0.0*	90.0 ± 0.3a	95.0 ± 0.2	60.0 ± 0.0*	65.0 ± 0.2	90.0 ± 0.3a	95.0 ± 0.0	95.0 ± 0.2	95.0 ± 0.2
	TP DTF Res- taurant	93.3 ± 0.2a	95.0 ± 0.2	65.0 ± 0.2*	55.0 ± 0.2*	100.0 ± 0.0a	100.0 ± 0.0	85.0 ± 0.2	100.0 ± 0.0	100.0 ± 0.0a	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
		76.7 ± 0.4a	80.0 ± 0.0	5.0 ± 0.2*	5.0 ± 0.2*	93.3 ± 0.2a	90.0 ± 0.0	25.0 ± 0.2*	25.0 ± 0.2*	96.7 ± 0.2a	100.0 ± 0.0	95.0 ± 0.2	65.0 ± 0.2*

Mean mortality followed by the different letters within the same row of the same population indicates that it is significantly different (Tukey's HSD; $P < 0.05$). Mean mortality with an asterisk within the same row of the same population indicates that it is significantly different from that of indoxacarb treatment only (Student's t -test; $P < 0.05$).

Table 6. Percentage mortality of field-collected German cockroaches after treatment with the diagnostic dose of imidacloprid (Im) and synergists

Strategy	Population	Mean % mortality \pm SEM											
		1x				3x				10x			
		Im only	Im + PBO	Im + DEM	Im + DEF	Im only	Im + PBO	Im + DEM	Im + DEF	Im only	Im + PBO	Im + DEM	Im + DEF
Baiting	EHI	100.0 \pm 0.0a	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0a	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0a	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0
	TC Da Res- taurant	36.7 \pm 0.2b	100.0 \pm 0.0*	80.0 \pm 0.0*	35.0 \pm 0.2	63.3 \pm 0.2b	100.0 \pm 0.0	85.0 \pm 0.2*	65.0 \pm 0.2	76.7 \pm 0.4b	100.0 \pm 0.0*	100.0 \pm 0.0*	70.0 \pm 0.4
	TC Jo Res- taurant	33.3 \pm 0.2b	100.0 \pm 0.0*	100.0 \pm 0.0*	15.0 \pm 0.2	93.3 \pm 0.2a	100.0 \pm 0.0	100.0 \pm 0.0	45.0 \pm 0.2*	100.0 \pm 0.0a	100.0 \pm 0.0	100.0 \pm 0.0	95.0 \pm 0.2
	TC 2T Res- taurant	73.3 \pm 0.2c	100.0 \pm 0.0*	20.0 \pm 0.4*	95.0 \pm 0.2*	96.7 \pm 0.2a	100.0 \pm 0.0	55.0 \pm 0.2*	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0
Random insecticide (RI)	TC Meat shop	76.7 \pm 0.2c	100.0 \pm 0.0*	75.0 \pm 0.2	85.0 \pm 0.2	100.0 \pm 0.0a	100.0 \pm 0.0	100.0 \pm 0.0	95.0 \pm 0.2	96.7 \pm 0.2a	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0
	TP Taipei	83.3 \pm 0.0a	100.0 \pm 0.0	15.0 \pm 0.2*	65.0 \pm 0.2	100.0 \pm 0.0a	100.0 \pm 0.0	80.0 \pm 0.0	90.0 \pm 0.0	100.0 \pm 0.0a	100.0 \pm 0.0	85.0 \pm 0.2	95.0 \pm 0.02
	TC Comes- table	33.3 \pm 0.4b	100.0 \pm 0.0*	55.0 \pm 0.2	40.0 \pm 0.0	63.3 \pm 0.4b	100.0 \pm 0.0*	95.0 \pm 0.2*	95.0 \pm 0.2*	93.3 \pm 0.2a	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0
	KS THSR	23.3 \pm 0.0b	80.0 \pm 0.0*	5.0 \pm 0.2	25.0 \pm 0.2	70.0 \pm 0.0b	100.0 \pm 0.0*	50.0 \pm 0.4	60.0 \pm 0.0	100.0 \pm 0.0a	100.0 \pm 0.0	75.0 \pm 0.2*	85.0 \pm 0.2
Insecticide rotation (IR)	KS Zuoying mall	33.3 \pm 0.2b	100.0 \pm 0.0*	45.0 \pm 0.2	35.0 \pm 0.2	73.3 \pm 0.2b	100.0 \pm 0.0*	70.0 \pm 0.0	76.0 \pm 0.2	100.0 \pm 0.0a	100.0 \pm 0.0	95.0 \pm 0.2	100.0 \pm 0.0
	KS Pizza mall	0.0 \pm 0.0d	35.0 \pm 0.2*	10.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0c	70.0 \pm 0.0*	25.0 \pm 0.2*	20.0 \pm 0.0	0.0 \pm 0.0c	95.0 \pm 0.2*	50.0 \pm 0.4*	35.0 \pm 0.2*
	TY CPSC	60.0 \pm 0.3c	100.0 \pm 0.0*	35.0 \pm 0.2	25.0 \pm 0.2*	73.3 \pm 0.2b	100.0 \pm 0.0*	55.0 \pm 0.2*	55.0 \pm 0.2*	90.0 \pm 0.0a	100.0 \pm 0.0	65.0 \pm 0.2*	75.0 \pm 0.2*
	TY EGSC	73.3 \pm 0.2c	100.0 \pm 0.0*	100.0 \pm 0.0*	70.0 \pm 0.4	100.0 \pm 0.0a	100.0 \pm 0.0	100.0 \pm 0.0	75.0 \pm 0.2	100.0 \pm 0.0a	100.0 \pm 0.0	100.0 \pm 0.0	100.0 \pm 0.0
Insecticide rotation (IR)	TP DTF Res- taurant	10.0 \pm 0.0d	85.0 \pm 0.2*	15.0 \pm 0.2	0.0 \pm 0.0	53.3 \pm 0.2b	95.0 \pm 0.2*	25.0 \pm 0.2*	10.0 \pm 0.0*	70.0 \pm 0.3b	100.0 \pm 0.0*	40.0 \pm 0.0*	35.0 \pm 0.2*

Mean mortality followed by the different letters within the same doses in deltamethrin treatment only significantly different (Tukey's HSD; $P < 0.05$).
Mean mortality with an asterisk within the same row of the same population indicates that it is significantly different from that of imidacloprid treatment only (Student's t -test; $P < 0.05$).

Table 7. Frequencies of *kdr* and *Rdl* mutations in susceptible and field collected German cockroach.

Population	#	<i>kdr</i>												<i>Rdl</i>					
		L993F						C764R						E434K			A302S		
		R/R	R/S	S/S	R/R	R/S	S/S	R/R	R/S	S/S	R/R	R/S	S/S	R/R	R/S	S/S	R/R	R/S	S/S
<i>Baiting</i>																			
TC Da Restaurant	10	3	5	2	0	0	0	10	0	0	10	0	0	0	10	10	0	0	0
TC Jo Restaurant	10	1	7	2	0	0	0	10	0	0	10	0	0	0	10	10	0	0	0
TC 2T Restaurant	10	0	1	9	0	0	0	10	0	0	10	0	0	0	10	0	3	7	0
TC Meat Shop	10	0	0	10	0	0	0	10	0	0	10	0	0	0	10	3	6	1	0
<i>Random insecticide (RI)</i>																			
TP Taipei	10	0	1	9	0	0	0	10	0	0	10	0	0	0	10	10	0	0	0
TC Comestible	10	1	4	5	0	0	0	10	0	0	10	0	0	0	10	10	0	0	0
KS THSR	10	0	0	10	0	0	0	10	0	0	10	0	0	0	10	10	0	0	0
KS Zuoying mall	10	4	5	1	0	0	0	10	0	0	10	0	0	0	10	10	0	0	0
KS Pizza mall	10	1	6	3	0	0	0	10	0	0	10	0	0	0	10	10	0	0	0
<i>Insecticide rotation (IR)</i>																			
TY CPCS	10	0	3	7	0	0	0	10	0	0	10	0	0	0	10	10	0	0	0
TY EGSC	10	0	3	7	0	0	0	10	0	0	10	0	0	0	10	10	0	0	0
TP DTF Restaurant	10	0	6	4	0	0	0	10	0	0	10	0	0	0	10	10	0	0	0
<i>Susceptible laboratory strain</i>																			
EHI	10	0	0	10	0	0	0	10	0	0	10	0	0	0	10	0	0	0	10

R/R: number of individuals homozygous for *kdr* mutation (considered to confer resistance to pyrethroids) and *Rdl* mutation (considered to confer resistance to fipronil).
 R/S: number of individuals heterozygous for point mutation.
 S/S: number of individuals with susceptible genotype.

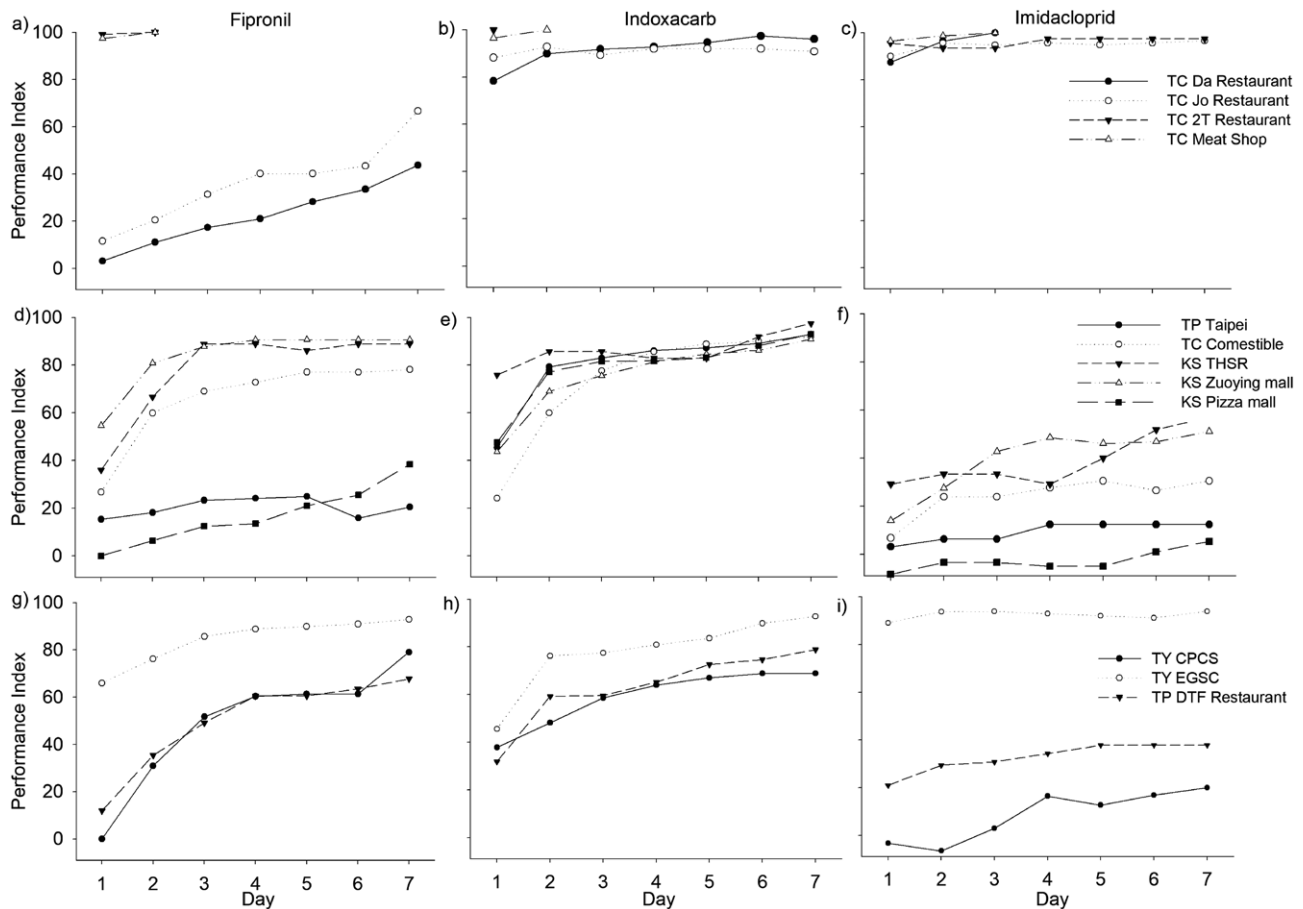


Fig. 1. Performance of Ultra Max (0.05% fipronil), Ke Mie Zhang (0.6% indoxacarb), and Premise Cockroach Gel (2.15% imidacloprid) against field-collected German cockroach populations under Baiting (a–c), random insecticide (RI) (d–f) and insecticide rotation management (IR) (g–i).

at $10 \times LD_{95}$, except for TP Taipei and TC Comestible under RI. These results contrast with [Pai et al. \(2023\)](#), who surveyed the resistance status of 5 field-collected populations from Southern Taiwan and reported no deltamethrin resistance. However, it is worth noting that the diagnostic dose used in the study was $22 \mu\text{g}/\text{male}$ ([Pai et al. 2023](#)), 70 times the highest dose used ($0.3 \mu\text{g}/\text{male}$) in the present study.

The extent of involvement of metabolic enzymes in deltamethrin resistance differs across various cockroach populations. The pretreatment of PBO significantly increased the mortality of some test populations from premises under RI and IR at $10 \times LD_{95}$, but the mortality did not exceed 60%. DEF and DEM synergists had no apparent effect on the deltamethrin resistance in these populations tested. The present results suggest the partial involvement of cytochrome P450 monooxygenases in deltamethrin resistance in these German cockroach populations. The contribution of esterases and glutathione S-transferases to deltamethrin resistance in the current test populations is limited. The result is in contrast with [Hu et al. \(2020\)](#), who examined the resistance status of German cockroaches collected from 24 premises in Taiwan and demonstrated the involvement of cytochrome P450 monooxygenases and esterases in conferring the deltamethrin resistance. Pyrethroid resistance involving these 2 detoxifying enzymes is common and has been reported from field populations in Thailand ([Tisgratog et al. 2023](#)), Iran ([Limoe et al. 2007](#)), Singapore ([Chai and Lee 2010](#)), Argentina ([Bone et al. 2022](#)), and US ([Lee et al. 2022](#)).

No C764R and E434K mutation was noted, as shown in other studies ([Devries et al. 2019](#), [Lee et al. 2022](#), [Liu et al. 2022](#), [Tisgratog](#)

[et al. 2023](#)). In contrast, the presence of the L993F *kdr* mutation in field populations from premises under various managements was mixed. For instance, our result showed that field-collected cockroaches from premises under the Baiting approach consisted of 2 susceptible populations (TC 2T Restaurant and TC Meat Shop), but the frequencies of the L993F mutation remained high in TC Da Restaurant and TC Jo Restaurant with 10%–30% being homozygous and 50%–70% being heterozygous. The result may indicate that these populations (TC Da Restaurant and TC Jo Restaurant) were undergoing a relaxation of selection pressure on pyrethroid resistance.

Those populations from premises under IR consisted of only heterozygous resistant and susceptible individuals. No homozygous resistant individual was present in the populations, indicating that the L993F mutation was not fixed in the populations. We infer that selection for the L993F mutation was relaxed under IR. However, the L993F mutation was not the sole cause of deltamethrin resistance, as the effect of the L993F mutation on sodium channel desensitization to pyrethroid was low ([Tan et al. 2002](#)). Enhanced cytochrome P450 monooxygenase activity resulting from other active ingredient pressure could have further conferred deltamethrin resistance in field-collected German cockroaches from premises under IR. The result is in accordance with [Fardisi et al. \(2019\)](#), who demonstrated 3 insecticide resistance intervention strategies, including using single active ingredient, mixture, and insecticide rotation treatments, that the latter strategy failed to reduce selection pressure on cyhalothrin due to cross-resistance among active ingredients.

By contrast, 3 field-collected populations from premises under RI consist of 10%–40% homozygous resistant and 10%–60% heterozygous resistant individuals. Coupled with the positive synergistic effect of PBO, the result suggests that combined metabolic and target-site resistance mechanisms may have conferred deltamethrin resistance in the field-collected cockroaches. In addition, for the KS THSR population under RI, almost all treated cockroaches survived the diagnostic doses at the highest dose tested, even under the pretreatment of synergists. None of the *kdr* mutations of the sodium channel was observed. The result suggests that other novel *kdr* point mutations not screened in the present study might be responsible for knockdown resistance toward pyrethroid insecticides in the German cockroach. This warrants further investigation.

Intensive use of fipronil gel bait potentially selected high frequencies of the *Rdl* gene mutation in field German cockroaches (Ang et al. 2013). Nevertheless, 83% of test populations from premises, irrespective of treatment regimes, survived upon exposure to fipronil at the dose of $3 \times LD_{95}$. *Rdl* mutation of the GABA-gated chloride channel was completely homozygous in all these field-collected populations, including populations under treatment regimes where fipronil gel bait was not used (Baiting and IR). The high frequency of A302S *Rdl* mutation of the GABA-gated chloride channel has also been reported in Thailand (Tisgratog et al. 2023), California (Lee et al. 2022), and North Carolina (Gonzalez-Morales et al. 2022).

Only 2 populations we evaluated from premises under the Baiting approach, i.e., TC 2T Restaurant and TC Meat Shop, had a low frequency of *Rdl* mutation of the GABA-gated chloride channel (0%–30% being homozygous resistant and 30%–60% being heterozygous resistant) and were moderately resistance (mortality ranged from 80% to 97% at $3 \times LD_{95}$). The result also indicates that the *Rdl* mutation alone does not necessarily elicit high fipronil resistance, but the combined effects of *Rdl* mutation and enhanced fipronil metabolism do (Scott and Wen 1997, Hansen et al. 2005, Kristensen et al. 2005, Gondhalekar and Scharf 2012, Ang et al. 2013, Lee et al. 2022). In particular, the high resistance levels to fipronil in TC Da Restaurant and TC Jo Restaurant populations under the Baiting approach may result from the combined elevated cytochrome P450 monooxygenases and *Rdl* mutation. No antagonistic effect of PBO on fipronil toxicity was found in the present study as opposed to the studies by Valles et al. (1997) and Ang et al. (2013).

Unlike populations under the Baiting approach, the pretreatment DEF and DEM significantly increased the mortality from 0% to 97% in fipronil-resistant populations under RI and IR. However, the effect of PBO on fipronil toxicity was negligible. The result indicates the major involvement of GST activity in fipronil metabolism. However, the role of esterases in fipronil metabolism requires further investigation due to the lack of ester-linkage in fipronil. The present finding opposed previous studies that enhanced oxidative and hydrolytic metabolism collectively enhanced fipronil resistance in the German cockroach (Lee et al. 2022, Tisgratog et al. 2023). We cannot precisely explain the difference in metabolic resistance observed between the Baiting approach and RI and IR. Still, the result suggests that the association between GST activity and detoxification of fipronil in German cockroaches appears to be population-specific, especially occurring only in populations managed by residual spraying with different active ingredients (RI and IR).

For fipronil bait evaluation, besides the 2 populations with low *Rdl* mutation frequency, which showed a PI of 100, there was no apparent pattern in PIs under different treatment regimes. However, it is interesting to observe that 4 out of 12 populations with combined

Rdl homozygous mutation and enhanced oxidative activity showed low to moderate PIs (20–67), while 5 out of 12 populations with combined *Rdl* homozygous mutation and mainly characterized with enhanced GST activity showed satisfactory control with moderate to high PIs (78–93).

For indoxacarb, 58% of field populations showed high mortality at the lowest dose ($1 \times LD_{95}$), and almost 80% of field populations exhibited over 90% mortality when exposed to the diagnostic dose of $3 \times LD_{95}$. The result suggests that the German cockroach populations in Taiwan may still be susceptible to indoxacarb. We did not observe any synergistic effect from PBO, DEF, and DEM synergists in most populations at the lowest dose ($1 \times LD_{95}$), indicating the lack of metabolism in reducing the susceptibility of Taiwanese German cockroaches to indoxacarb. On the contrary, most effects were antagonistic at low doses as supported by Gondhalekar et al. (2016). By contrast, DEM and DEF synergists synergized the toxicity of indoxacarb in TC Comestible under RI. The results of the topical bioassay are in accordance with the PI of indoxacarb-containing bait in the present study, registering the highest PIs among the baits tested.

Our finding indicates that partial mortality from topically applied imidacloprid occurred across all field populations regardless of the treatment regimes. Increased imidacloprid tolerance is mainly due to enhanced oxidative activity in detoxifying imidacloprid. In addition, elevated hydrolytic and GST activity also play a minor role in negating imidacloprid resistance in some Taiwanese populations under the 3 management strategies. Among the population tested, the PIs of imidacloprid-containing bait were relatively higher in populations under the Baiting approach (97–100) compared to the other populations from RI (5–57) and IR (20–94). We suspect that the discontinued use of pyrethroid treatment in premises under the Baiting approach may have mitigated the oxidative activity in the populations, thus increasing the overall PIs of imidacloprid bait. Notably, 2 field German cockroach populations (TP Taipei and KS Pizza mall) exposed to imidacloprid bait showed PIs of 5–12 on day 7 with reduced mortality recorded, indicating bait palatability issue. The cockroaches likely avoided the imidacloprid bait due to aversion to bait ingredients (Silverman and Bieman 1993, Appel and Rust 2021).

In summary, the study demonstrated that different treatment regimes can influence the frequency of L993F *kdr* mutation and detoxifying enzymes involved in deltamethrin resistance. Populations in premises under the Baiting approach and IR were probably undergoing a relaxation of selection pressure on pyrethroid resistance as evidenced from reduced homozygosity of the L993F *kdr* mutation in the populations. Treatment regimes are important in determining the PI of fipronil- and imidacloprid-containing bait. Our result does not support the notion that using multiple AIs, either through RI or IR, could reduce the frequency of the *Rdl* mutation. However, it might influence detoxifying enzyme profiles, determining the fipronil sensitivity. In particular, fipronil resistance involving cytochrome P450 monooxygenase substantially reduced the efficacy of fipronil bait. In contrast, populations with enhanced GST activities showed effective control when exposed to fipronil bait. Indoxacarb could be an alternative to ensure satisfactory management against resistant German cockroaches in Taiwan. Although increased imidacloprid resistance in most populations is mainly induced by enhanced oxidative activity, the results do not directly correlate with imidacloprid bait performance. Instead, the treatment regimes matter—imidacloprid bait has proven effective when the pyrethroid pressure is withdrawn. However, it is essential to acknowledge the potential caveat because of the lack of information about

the retrospective resistance level of German cockroaches before the current management programs were conducted and how long these treatment programs were conducted, which may confound the present result.

Supplementary data

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

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Author contributions

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