

## Household and Structural Insects

# Association of *CYP4G19* Expression With Gel Bait Performance in Pyrethroid-Resistant German Cockroaches (Blattodea: Ectobiidae) From Taiwan

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### Abstract

Overexpression of a cytochrome P450 gene, *CYP4G19*, is known to associate with pyrethroid resistance in the German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae). In this study, we investigated the *CYP4G19* expression level in 20 field-collected strains of *B. germanica* in Taiwan. We also examined the level of adult male susceptibility to imidacloprid, fipronil, indoxacarb, and hydramethylnon using single-diagnostic dose bioassays and their susceptibility to corresponding gel baits to determine how the *CYP4G19* expression level influences the cockroach gel bait performance. Results showed that the *CYP4G19* gene expression level among the field-collected German cockroach was 1.8- to 9.7-fold higher than that of the susceptible strain. It was negatively correlated ( $P < 0.05$ ) with the % mortality after treatments with imidacloprid and fipronil diagnostic doses. However, no correlation was found between *CYP4G19* gene expression with the % mortality after treatment with indoxacarb and hydramethylnon diagnostic doses. Indoxacarb and hydramethylnon baits showed high efficacy against the field strains with a mean mortality of  $97.58 \pm 1.35\%$  and  $90.95 \pm 1.65\%$ , respectively. This study provided the first evidence of cross-resistance to imidacloprid and fipronil in pyrethroid-resistant German cockroaches due to overexpression of *CYP4G19*.

**Key words:** cytochrome P450 monooxygenase, metabolic resistance, pyrethroid, insecticide resistance management

The German cockroach, *Blattella germanica* (L.), is an important urban and public health pest (O'Connor and Gold 1999, Gore and Schal 2007). Chemical control remains the most effective method to manage German cockroach infestation in low-income housing, hotels, apartments, and commercial kitchens (Peterson and Shurdut 1999, Wang and Bennett 2006). However, heavy reliance and injudicious use of insecticides, coupled with a short life-cycle and high adaptability, have led to the development of insecticide resistance in *B. germanica* (Fardisi et al. 2019, Tang et al. 2019).

Insect P450s play a significant role in physiological pathways, including the biosynthesis and degradation of pheromones, ecdysteroids, and juvenile hormone (Reed et al. 1994, Sutherland et al. 1998, Winter et al. 1999). Insect P450s are also implicated in detoxification, resulting in insecticide resistance in insects (Feyereisen 1999, Scott 1999). In *B. germanica*, a total of 163 P450 genes were putatively identified through transcriptome sequencing (Zhou et al.

2014). A few P450s are involved in insecticide resistance, for example, *CYP6L1* (Wen and Scott 2001a), *CYP6K1*, *CYP6J1* (Wen and Scott 2001b), and *CYP4G19* (Pridgeon et al. 2003, Guo et al. 2010). The overexpression of *CYP4G19* gene was associated with detoxification of pyrethroids (Pridgeon et al. 2003, Guo et al. 2010) and cuticular penetration resistance via biosynthesis of hydrocarbons in resistant insects (Chen et al. 2020).

Residual sprays of pyrethroids such as deltamethrin, lambda-cyhalothrin, cyphenothrin, cypermethrin, tetramethrin, etofenprox, and cyfluthrin remain the mainstay of German cockroach control in Taiwan. However, pyrethroid resistance due to cytochrome P450 monooxygenase is widespread among field populations of *B. germanica* (Hu et al. 2020). In most situations, pest management professionals resort to gel baits whenever they experience control failure with pyrethroid sprays. It was also observed that cytochrome P450 monooxygenase could potentially render fipronil, imidacloprid,

and indoxacarb gel baits ineffective in these pyrethroid-resistant strains (Hu et al. 2020). However, the result was inconclusive as some gel baits tested had low palatability. While there are studies that demonstrated the influence of cytochrome P450 monooxygenase in conferring resistance to fipronil, imidacloprid, and indoxacarb in German cockroaches (Wei et al. 2001, Gondhalekar and Scharf 2012, Gondhalekar et al. 2016), there are others that reported otherwise (Holbrook et al. 2003, Limoe et al. 2007, Wu and Appel 2017). It is worth noting that most of these studies drew their conclusions based on synergism studies using piperonyl butoxide (PBO). Given that many field populations of *B. germanica* are deltamethrin-resistant (Hu et al. 2020), investigation on the relationship between *CYP4G19* gene expression levels of the field strains and these bait toxicants' resistance levels may help resolve these conflicting findings.

A good understanding of resistance and cross-resistance patterns in field-collected German cockroaches is necessary to successfully implement insecticide rotation with different modes of action to delay insecticide resistance development. Besides, it is also important to know whether the cross-resistance could affect commercial gel baits' performance and whether the cross-resistance effect may be mitigated if a bait is highly palatable. This study examined the *CYP4G19* gene expression levels in the 20 field-collected strains of *B. germanica*. We also tested the field strains with the diagnostic doses of fipronil, imidacloprid, hydramethylnon, and indoxacarb, and with the commercial gel baits of the said insecticides. Lastly, we determined the relationship between the *CYP4G19* gene expression levels with the cockroach mortality after tested with diagnostic doses of the insecticides.

## Materials and Methods

### Insects

Twenty field populations of *B. germanica* were collected from infested premises in 2017–2018 (Hu et al. 2020). These premises were regularly treated using residual sprays of pyrethroids (e.g., deltamethrin). Whenever there is a callback or control failure, the pest control operators will use gel baits to treat the cockroaches. Compared with susceptible laboratory strain, deltamethrin resistance ratio ranged from 1.5 to 817.5. Of the 20 field strains tested, the resistance levels of two strains (TC Supermarket and TC THSR) failed to be negated by both PBO and DEF, indicating that other mechanisms are involved in the resistance, including *kdr* resistance. Their pyrethroid resistance profiles have been described in Hu et al. (2020). A susceptible laboratory strain (EHI) (see Chai and Lee 2010, Ang et al. 2013) that has been reared in the laboratory without any insecticide exposure for more than 40 yr, was used for comparison. All insects were reared in round polyethylene containers (24 cm diameter × 32 cm height) with dog food (Perfect Companion Group Co., Ltd., Hsinchu, Taiwan), water, and corrugated cardboard ad libitum, and environmental conditions of 25 ± 1°C, 50 ± 5% RH, and a photoperiod of 12:12 (L:D) h.

### Insecticides

We used technical grade insecticide imidacloprid (94%, Tagros Chemicals India Ltd., Chennai, India), hydramethylnon (98%, Kukbo Science Co., Ltd., Cheongju-si, Korea), fipronil (98%, Chem Service, Inc., West Chester, PA), and indoxacarb (96%, Sigma-Aldrich Corporation, Saint Louis, MO) for topical bioassays. All chemicals were dissolved in absolute acetone (Union Chemical Works Ltd., Hsinchu, Taiwan) as stock solutions and stored at 4°C until use. The stock solutions were serially diluted using acetone to the designated concentrations before topical application.

### Topical Bioassay

Adult males were collected from the rearing container and acclimatized in a polyethylene cup with dog food and water 24 h before topical bioassay. Ten males were immobilized with ice for approximately 2 min. One microliter of the diagnostic dose (LD<sub>95</sub>) of an insecticide (Chai and Lee 2010, Supp Tables S1–S4 [online only]) generated earlier using the EHI-susceptible strain was topically applied onto each cockroach's first abdominal sternite using a handheld microapplicator (Burkard Scientific Ltd., Middlesex, United Kingdom). The treated individuals were then transferred into a clean glass jar (base area = 28.27 cm<sup>2</sup>, height = 9 cm) with food and water. The experiment was replicated 5× for each strain. Mortality of test insects (DD mortality) was scored at 48 h for treatment of imidacloprid, fipronil, and indoxacarb, and 72 h for hydramethylnon.

### Commercial Gel Bait Formulation Assays

Four commercial gel baits were evaluated against all field and susceptible strains of *B. germanica*. The tested baits were Premise Cockroach Bait (containing 2.15% imidacloprid) (Bayer AG, Petaling Jaya, Malaysia), Jin-Li-Hai Ultra Max (containing 0.05% fipronil) (Kukbo Science Co., Ltd., Cheongju-si, Korea), Advion Cockroach Gel Bait (containing 0.6% indoxacarb) (Syngenta AG, Singapore), and Siege Cockroach Gel (containing 2.15% hydramethylnon) (BASF Taiwan Ltd., Taiwan). The test procedures followed essentially that described in Hu et al. (2020). Briefly, the adult males were acclimatized with water only for 48 h. Ten adult males were introduced into a polyethylene container (36 × 28 × 12 cm) and provided with dog food, harborage, water, and 1 g gel bait ad libitum. Mortality and bait consumption was scored daily up to 7 d or when 100% mortality was achieved. For all strains, four baits were tested, and the experiment was replicated 4×.

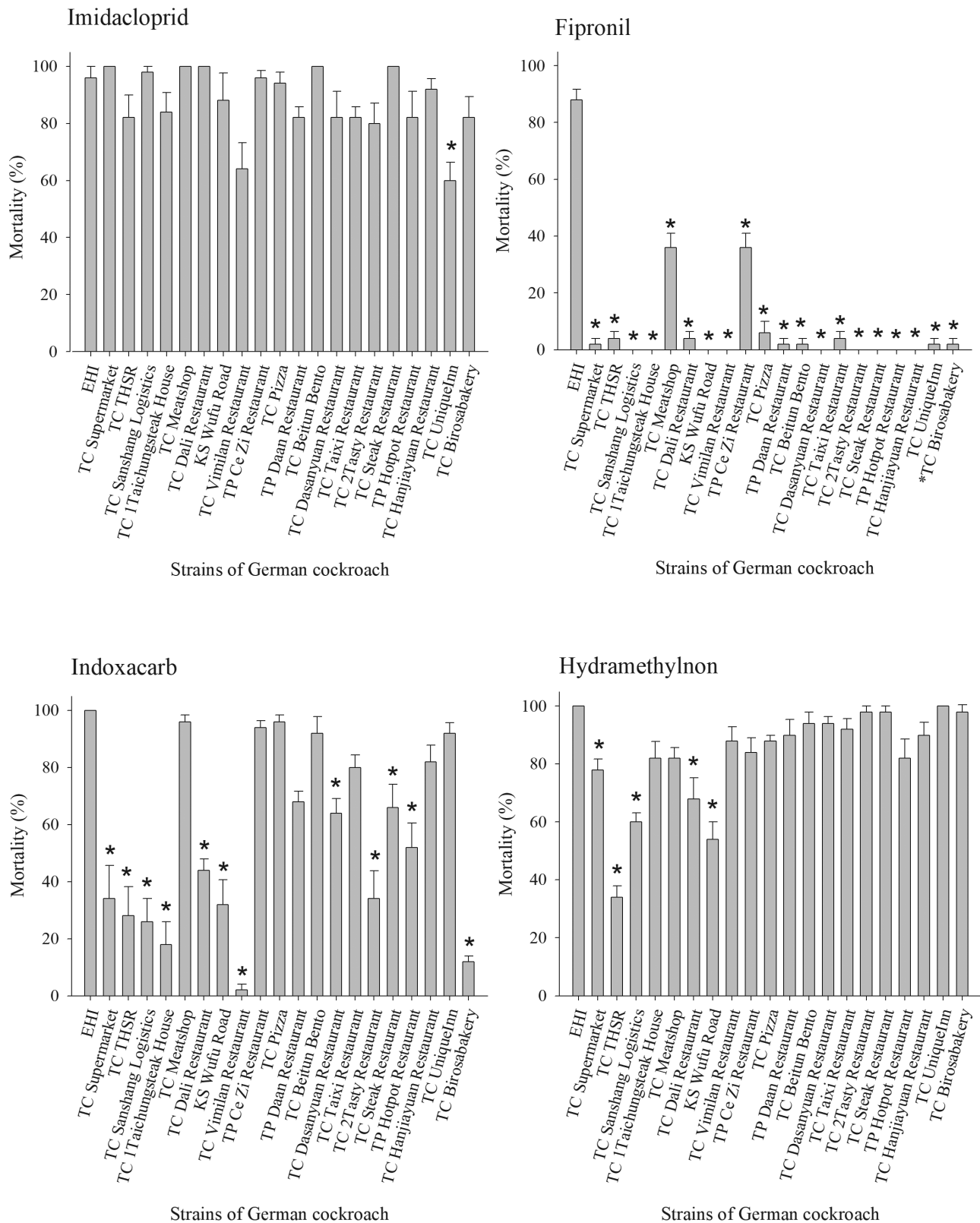
### RNA Extraction and cDNA Preparation

Total RNAs were extracted with TRIzol Reagent (Invitrogen, Carlsbad, CA) and PureLink RNA Mini Kit (Invitrogen) using whole body of adult male *B. germanica*. RNase-Free DNase I (Lucigen, Middleton, WI) was used to digest genomic DNA. All RNA samples were stored at -80°C until use. In total, seven male cockroaches of each field population were prepared for cDNA synthesis. RNA quality and quantity were determined using the Nanodrop ND-2000 (Thermo Scientific, Wilmington, DE). The cDNA was run with 1 µg of total RNA in a 20 µl reaction volume using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). All methods followed the manufacturer's protocol.

### Determination of *CYP4G19* Gene Expression Levels of Susceptible and Field Populations by Real-Time Quantitative PCR

The expression of the *CYP4G19* gene (AY176056, GenBank) was examined. The primers (Supp Table S4 [online only]) amplified a 187-bp fragment from *CYP4G19* mRNA. The housekeeping gene, *Actin5C* (AJ862721, GenBank), yielding a 213-bp fragment, was used as an internal control.

Real-time quantitative PCR was performed using CFX Connect Real-Time System (Bio-Rad) with iQ SYBR Green Supermix (Bio-Rad). The reaction mixtures contained 10 µl of 2× SYBR Green Supermix, 0.4 µl each of 10 µM for forward and reverse primers, 1 µl of cDNA template, and 8.6 µl DNase-free water. Each run contained a control with no template cDNA. The PCR consisted of 94°C for 5 min and 40 cycles at 94°C for 30 s, 61.4°C for 10 s, and 72°C for



**Fig. 1.** Percentage of mortality of adult males of the field strains of *B. germanica* after being treated with the diagnostic dose of insecticides using topical assay method. The asterisk (\*) indicates significant difference ( $P < 0.05$ ) using Student's *t*-test when compared with EHI.

10 s. Then, the PCR products were subjected to melting temperatures ranging from 65 to 95°C with increasing to 0.5°C per 5 s to generate a melting curve for examining specific amplification of target genes.

The expression level and fold change of target genes in resistance strains relative to the susceptible strain were calculated by referring to the comparative  $C_T$  method (Schmittgen and Livak

**Table 1.** Percentage of mortality of adult males of the field strains of *B. germanica* after treatment with gel baits for 7 d

Strains	Premise (imidacloprid)	Jin-Li-Hai Ultra Max (fipronil)	Advion (indoxacarb)	Siege (hydramethylnon)
	Mortality (SE)	Mortality (SE)	Mortality (SE)	Mortality (SE)
EHI	95.64% (2.52)	100.00% (0.00)	100.00% (0.00)	100.00% (0.00)
TC Supermarket	88.64% (8.60)	97.92% (2.08)	100.00% (0.00)	93.33% (6.67)
TC THSR	29.77% (11.83)*	52.39% (2.62)*	72.63% (5.20)*	93.33% (3.33)
TC Sanshang Logistics	51.11% (3.73)*	77.64% (2.80)*	97.92% (2.08)	93.33% (6.67)
TC 1Taichungsteak House	63.26% (7.12)*	75.00% (8.60)*	97.92% (2.08)	96.67% (3.33)
TC Meatshop	93.18% (6.82)	100.00% (0.00)	100.00% (0.00)	83.33% (12.02)
TC Dali Restaurant	92.45% (2.55)	50.76% (6.47)*	95.45% (2.62)	100.00% (0.00)
KS Wufu Road	63.64% (6.93)*	90.24% (5.34)	100.00% (0.00)	90.00% (5.77)
TC Vimilan Restaurant	22.16% (4.39)*	45.83% (5.83)*	95.83% (2.41)	93.33% (3.33)
TP Ce Zi Restaurant	62.12% (10.79)*	97.73% (2.27)	100.00% (0.00)	96.67% (3.33)
TC Pizza	79.23% (4.97)*	100.00% (0.00)	100.00% (0.00)	76.67% (14.53)
TP Daan Restaurant	79.55% (5.72)*	69.39% (10.39)*	100.00% (0.00)	100.00% (0.00)
TC Beitun Bento	91.48% (5.90)	95.83% (4.17)	97.73% (2.27)	90.00% (5.77)
TC Dasanyuan Restaurant	65.23% (13.77)*	36.39% (13.81)*	97.73% (2.27)	93.33% (6.67)
TC Taixi Restaurant	97.73% (2.27)	100.00% (0.00)	100.00% (0.00)	93.33% (3.33)
TC 2Tasty Restaurant	84.95% (5.00)	15.91% (10.74)*	97.73% (2.27)	83.33% (6.67)
TC Steak Restaurant	62.12% (5.87)*	97.73% (2.27)	100.00% (0.00)	90.00% (5.77)
TP Hotpot Restaurant	61.36% (7.76)*	93.18% (6.82)	97.73% (2.27)	80.00% (5.77)*
TC Hanjiayuan Restaurant	28.06% (4.52)*	70.37% (5.67)*	100.00% (0.00)	93.33% (3.33)
TC UniqueInn	71.21% (6.60)*	79.32% (14.50)	100.00% (0.00)	96.67% (3.33)
TC Birosabakery	69.55% (8.08)*	97.73% (2.27)	100.00% (0.00)	73.33% (12.02)
Mean	69.04% (4.86)	76.21% (5.36)	97.58% (1.35)	90.95% (1.65)

The asterisk (\*) indicates significant difference ( $P < 0.05$ ) using Student's *t*-test when compared with EHI.

**Table 2.** The correlation between mortality percentages of field strains of *B. germanica* after treatment of diagnostic dose and gel baits, and their *CYP4G19* expression levels

Insecticides	df	F	R <sup>2</sup>	P
The correlation between DD mortality and bait mortality				
Imidacloprid	20	2.99	0.14	0.10
Fipronil	20	1.94	0.16	0.19
Indoxacarb	20	0.98	0.05	0.34
Hydramethylnon	20	0.18	0.01	0.67
The correlation between <i>CYP4G19</i> expression level and DD mortality				
Imidacloprid	20	6.13	0.24	0.02
Fipronil	20	5.36	0.22	0.03
Indoxacarb	20	0.73	0.04	0.40
Hydramethylnon	20	1.63	0.08	0.22

2008) after normalizing with the housekeeping gene *Actin5C* ( $C_T$  mean =  $23.07 \pm 0.13$  [ $\pm$  SE]).

### Data Analysis

We used Student's *t*-test to analyze the differences of mortality among strains and the EHI-susceptible strain in topical bioassay and commercial gel bait formulation assays. The relationships between the expression levels of *CYP4G19* gene, and DD and bait mortality, and between DD mortality and bait mortality were analyzed using linear regression analysis. The value was subjected to log transformation before analyses. All analyses in this study were performed in SPSS analysis version 11.0 (SPSS Inc., Chicago, IL).

## Results

### Topical Bioassay Using Diagnostic Doses

All field strains of *B. germanica* show high mortality when treated with imidacloprid (82–100%) which did not differ significantly from

that of the EHI-susceptible strain, except the TC Uniqueinn, TC Vimilan Restaurant, TP Daan Restaurant, and TC Taixi Restaurant strains (Fig. 1; Supp Table S5 [online only]). On the contrary, 20 field strains tested with fipronil demonstrated significantly lower mortality (0–36%) than the EHI strain ( $88.0 \pm 3.7\%$ ) (Fig. 1; Supp Table S5 [online only]). When tested with indoxacarb, seven strains registered 40–90% mortality after indoxacarb treatment while another eight strains had <40% mortality. The % mortalities of 15 strains were significantly different from that of the EHI strain. The diagnostic dose of hydramethylnon resulted in >90% mortality in nine field strains which were statistically similar to that of the EHI strain. One strain, TC THSR, only recorded  $34.0 \pm 8.9\%$  mortality (Fig. 1; Supp Table S5 [online only]).

### Commercial Gel Bait Formulation Assays and Their Association With the Toxicity of Each Insecticide

When tested with Premise and Jin-Li-Hai, more than 50% of the field strains showed mortality significantly lower than that of the

**Table 3.** Relative expression levels of *CYP4G19* gene among field strains of *B. germanica* and that of the EHI-susceptible strain

Strains	$\Delta$ Ct (SE)	$\Delta\Delta$ Ct	FC
EHI	5.52 (0.62)		
TC Supermarket	3.65 (0.64)	-1.87	3.66
TC THSR	3.24 (0.47)*	-2.28	4.87
TC Sanshang Logistics	3.45 (0.35)*	-2.07	4.21
TC 1Taichungsteak House	3.68 (0.69)	-1.84	3.59
TC Meatshop	4.68 (0.38)	-0.84	1.79
TC Dali Restaurant	4.21 (0.58)	-1.32	2.49
KS Wufu Road	3.12 (0.40)*	-2.40	5.28
TC Vimilan Restaurant	2.96 (0.41)*	-2.56	5.89
TP Ce Zi Restaurant	2.96 (0.86)*	-2.57	5.92
TC Pizza	3.06 (0.51)*	-2.46	5.49
TP Daan Restaurant	2.83 (0.33)*	-2.70	6.48
TC Beitun Bento	3.42 (0.56)*	-2.10	4.28
TC Dasanyuan Restaurant	3.93 (0.20)*	-1.59	3.00
TC Taixi Restaurant	3.19 (0.40)*	-2.33	5.03
TC 2Tasty Restaurant	2.24 (0.21)*	-3.28	9.73
TC Steak Restaurant	2.68 (0.39)*	-2.84	7.16
TP Hotpot Restaurant	3.52 (0.23)*	-2.00	4.01
TC Hanjiayuan Restaurant	2.79 (0.34)*	-2.73	6.64
TC UniqueInn	2.38 (0.34)*	-3.14	8.80
TC Birosabakery	2.28 (0.18)*	-3.24	9.44

Fold change (FC),  $2^{-(\Delta\Delta\text{Ct})}$ ;  $\Delta\Delta\text{Ct}$ , field strain  $\Delta\text{Ct}$  - EHI  $\Delta\text{Ct}$ . The asterisk (\*) indicates significant difference ( $P < 0.05$ ) using Student's *t*-test when compared with EHI.

EHI strain (Table 1). On the contrary, Advion and Siege caused high mortality with no significant difference in most field strains when compared with EHI (Table 1). No significant correlation between DD and bait mortality was detected, suggesting that other factors such as bait palatability could be involved (Table 2). The total bait consumption varied with the bait types (mean bait consumption: imidacloprid:  $85.60 \pm 8.85$  mg; fipronil:  $172.41 \pm 19.71$  mg; indoxacarb:  $416.15 \pm 32.53$  mg; hydramethylnon:  $31.61 \pm 20.67$  mg).

### The Expression Level of *CYP4G19* Gene and Its Association With the Toxicity of Each Insecticide

The expression levels of *CYP4G19* gene in the field strains ranged from 1.8- to 9.7-fold when compared to that of the EHI strain (Table 3). The correlations between DD mortality of imidacloprid and fipronil and the expression level of *CYP4G19* gene were significant ( $P < 0.05$ ) (Fig. 2; Table 2). In contrast, no significant correlation was found between the expression of *CYP4G19* gene and DD mortality of indoxacarb and hydramethylnon in field-collected German cockroach (Fig. 2; Table 2).

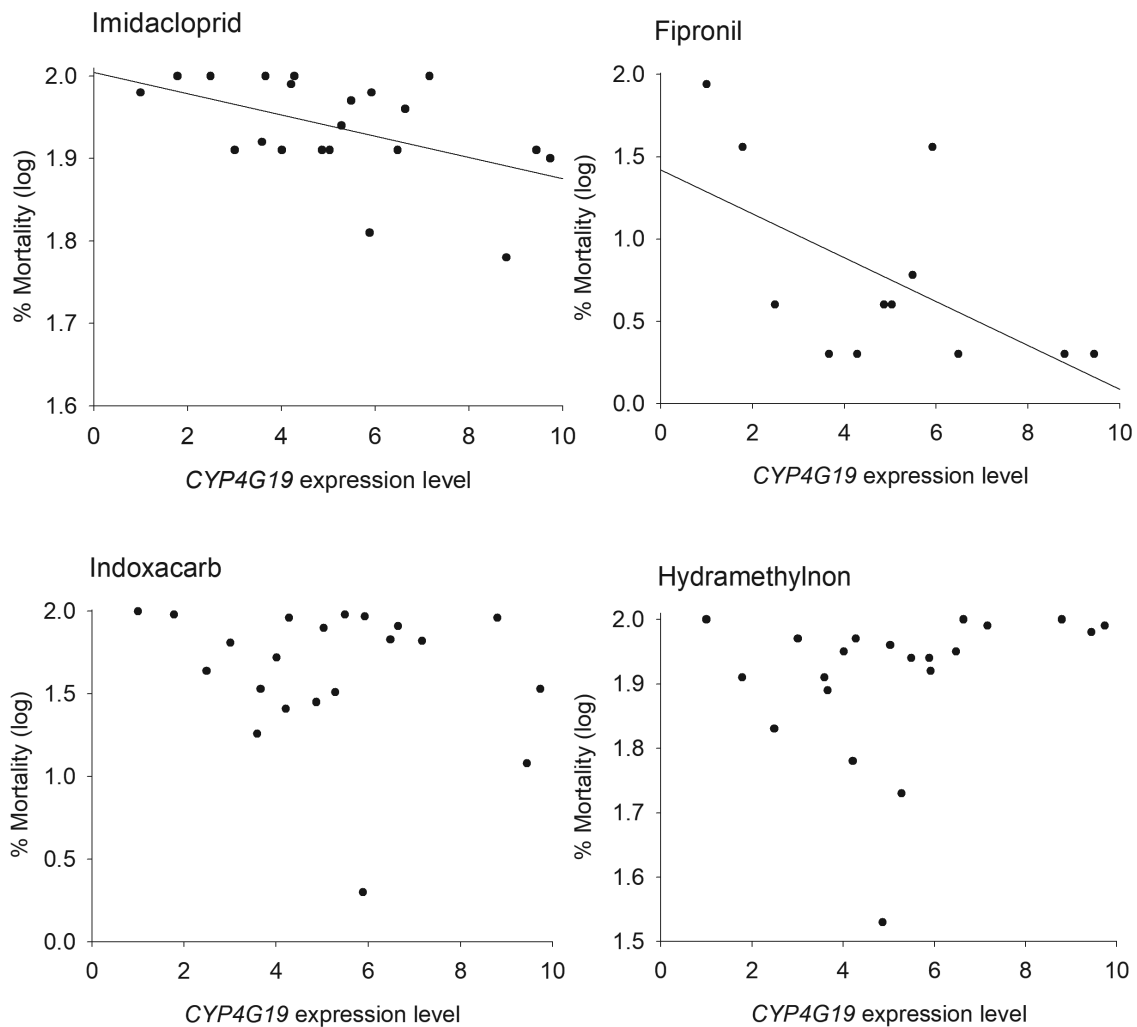
## Discussion

*CYP4G19* gene's expression levels in the field-collected pyrethroid-resistant strains of the German cockroach increased 1.8- to 9.7-fold relative to the susceptible strain and was significantly correlated with the mortality from imidacloprid DD treatment. This cross-resistance to imidacloprid reduced the performance of imidacloprid gel baits against some strains tested in this study. In *Drosophila*, one of cytochrome P450 genes, *CYP6G1* overexpression, also conferred cross-resistance to imidacloprid (Daborn et al. 2001). The cytochrome P450 monooxygenase involvement in imidacloprid metabolism was observed in brown planthopper (Zewen et al. 2003), and house flies

(Liu and Yue 2000). However, other studies on pyrethroid-resistant German cockroaches showed contradictory results that P450 monooxygenase-mediated detoxication was not the mechanism responsible for cross-resistance to imidacloprid (Wen and Scott 1997, Wei et al. 2001). In other words, the same mechanisms will not evolve in the same manner for all populations. Mutations and selection pressures differentially shape resistance evolution in different populations.

Fipronil is a popular toxicant in gel bait formulation for the management of German cockroaches because of its fast-action (Kaakeh et al. 1997, Holbrook et al. 2003), besides other advantages, such as longer residual activity, safer application, and low environmental impact (Reierson 1995, Buczkowski and Schal 2001). In this study, the German cockroach field strains showed low mortality ( $8.95 \pm 4.57\%$ ) after treatment with fipronil DD. The low mortality after DD treatment also was significantly correlated with the increased expression level of the *CYP4G19* gene. Our study suggested cross-resistance to fipronil in pyrethroid-resistant German cockroaches through overexpression of *CYP4G19*. This cross-resistance significantly reduced the efficacy of fipronil gel bait in the present study. The results indirectly concur with that reported in Gondhalekar and Scharf (2012), that pretreatment of PBO (monooxygenase inhibitor) significantly reduced the magnitude of fipronil resistance in a field-collected strain. On the contrary, some studies showed that pyrethroid resistance in the German cockroach did not predispose them to be resistant to fipronil (Wei et al. 2001, Holbrook et al. 2003, Wu and Appel 2017). It is worth noting that the enhanced oxidative activity may not always reduce the toxicity of fipronil but may also elevate fipronil toxicity (Valles et al. 1997, Ang et al. 2013). This is because the toxic metabolite fipronil sulfone can be catalyzed by both cytochrome P450-mediated microsomal monooxygenase and glutathione S-transferases (GSTs) in insects (Scharf et al. 2000, Zhao et al. 2005).

Indoxacarb DD treatment on the field strains suggested that 15 strains may be resistant to indoxacarb. However, *CYP4G19* is unlikely related to indoxacarb resistance as no significant association between the expression level of the *CYP4G19* gene and the % mortality after indoxacarb DD treatment was found. Nevertheless, there is a possibility that other forms of P450s may be involved. Gondhalekar et al. (2016) demonstrated that P450 activity of *B. germanica* was directly involved in indoxacarb resistance by multiple strains. Similarly, Sayyed et al. (2014) reported that the increased microsomal oxidase activity conferred indoxacarb resistance in *Spodoptera litura* (Fabricius). Besides, other metabolic resistance mechanisms such as elevated esterases may be involved. Hu et al. (2020) earlier found that elevated esterases were also associated with deltamethrin resistance in most strains of TC Sanshang Logistics, TC 1Taichungsteak House, TC Dali Restaurant, KS Wufu Road, TC Vimilan Restaurant, TP Daan Restaurant, TC 2Tasty Restaurant, TC Steak Restaurant, and TP Hotpot Restaurant. Similarly, Sayyed and Wright (2006) demonstrated that indoxacarb-resistant in a diamondback moth strain, *Plutella xylostella* L. was due to elevated esterase enzyme. Besides, Hu et al. (2020) also documented the involvement of other resistance mechanisms such as target site insensitivity and/or GST. A combination of GST, carboxylesterase, and *kdr* resistance (L1014F) was reported to cause indoxacarb resistance in the beet armyworm, *Spodoptera exigua* (Hübner) (Gao et al. 2014). All these possibilities, however, warrant further investigation. Despite this, indoxacarb baits showed significant mortality against most *B. germanica* strains in this study. The indoxacarb bait consumption rate was 2–13× higher than that of the other gel baits. The



**Fig. 2.** The correlation between *CYP4G19* expression level and mortality percentages of field strains of *B. germanica* from Taiwan after treatment with diagnostic dose.

higher consumption of bait likely negated the effects of resistance to indoxacarb, or indoxacarb could be more toxic when ingested (Gondhalekar et al. 2013) compared to contact.

The field strains showed high mortality when treated with hydramethylnon DD. A mere  $31.61 \pm 20.67$  mg of gel bait was sufficient to cause at least 73% mortality of the German cockroach strains. Hydramethylnon showed significant mortality against the pyrethroid resistance populations in the present study and other studies (Appel 1990, Lee 1998). However, the hydramethylnon bait should only be employed sparingly as the development of cross-resistance is possible. Single and repeated use of hydramethylnon potentially prolongs the resistant cockroach's survival time and diminishes the secondary killing effect via excreta ingestion (Ko et al. 2016).

In summary, overexpression of *CYP4G19* gene was associated with cross-resistance to imidacloprid and fipronil in field strains of *B. germanica* in this study. Indoxacarb and hydramethylnon baits caused significant mortality against these field strains in laboratory assays. To manage these cockroach populations effectively, we recommend quarterly rotation between indoxacarb and hydramethylnon baits and avoid the use of residual pyrethroid sprays.

### Supplementary Data

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

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