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ASSESSMENT OF INSECTICIDE RESISTANCE RISK TO COMMERCIAL GEL BAIT IN FIELD PYRETHROID-RESISTANT GERMAN COCKROACHES

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Abstract Insecticide resistance poses an ongoing challenge in controlling German cockroach infestations, *Blattella germanica* (L.) (Blattodea: Ectobiidae), with reports of resistance to 45 active ingredients documented. Baiting is an effective control measure to suppress the German cockroach population in Taiwan. However, increasing reports on their ineffectiveness in reducing cockroach population due to cross-resistance involving cytochrome P450 monooxygenase. In the present study, we tested three field populations of German cockroaches, which are characteristic of being susceptible to deltamethrin but resistant to fipronil (WF), low resistance to deltamethrin and fipronil (CR), and high resistance to deltamethrin and fipronil (BZ). The test cockroaches were subjected to fipronil-, imidacloprid- and indoxacarb-containing commercial gel baits. The surviving cockroaches were reared for the next generations. The lethal dose causing 50% population mortality was generated. The values of LD_{50} from each population under respective treatments were compared with laboratory susceptible strain (EHI) to generate a resistance ratio (RR_{50}). The result showed that the RR_{50} for fipronil in WF and BZ remained high throughout generations, with RR_{50} ranging from 8.9 to 25.8. Increasing RR_{50} (1.4 – 5.0) was also observed in CR. In contrast, a subtle or gradual increment in RR_{50} was observed under imidacloprid and indoxacarb gel bait treatment in test populations irrespective of resistance status. In conclusion, the resistant status is essential in determining the performance of fipronil-containing bait. To productively manage the German cockroach populations, indoxacarb baits could be an alternative to ensure satisfactory management against resistant German cockroaches in Taiwan.

Key words German cockroach, insecticide resistance, bait selection, realized heritability

INTRODUCTION

Insecticide resistance caused by the heavy reliance on and frequent use of insecticides poses an ongoing challenge in the management of the German cockroach, *Blattella germanica* (Hu et al., 2020; Scharf and Gondhalekar, 2021; Tisgratog et al., 2023). To date, *B. germanica* has been reported to develop resistance toward 45 insecticides, so making decisions regarding managing *B. germanica* has become essential (Mota-Sanchez and Wise, 2025).

In Taiwan, baiting has been regarded as an effective and popular method for managing *B. germanica* since the introduction of commercial gel baits containing fipronil in the 2000s (Kruaysawat et al., 2024). However, increasing complaints from pest management professionals have highlighted the ineffectiveness of gel baits in reducing the cockroach population. Previous studies have also indicated that the failure of baiting in cockroach management is attributed to cross-resistance involving cytochrome P450 monooxygenase (Hu et al., 2020; 2021). Given the potential risk of reduced bait efficacy, it is essential to assess the development of insecticide resistance to gain deeper insights into the durability and reliability of insecticides.

In this study, we evaluated three field populations of *B. germanica*, which are characteristic of being susceptible to deltamethrin but resistant to fipronil (WF), low resistance to deltamethrin and fipronil (CR), and high resistance to deltamethrin and fipronil (BZ), as reported to Hu et al. (2020; 2021). With commercial bait selection and profiling the toxicity data of fipronil, imidacloprid, and indoxacarb, the development of resistance of these insecticides in BZ, CR, and WF was quantified and visualized as resistance ratio (RR) and realized heritability (h^2).

MATERIALS AND METHODS

Insect collection and rearing Three field populations of *B. germanica* (TP Breeze Mall, BZ; TC Carrefour, CR; KS Wufu, WF) were collected from infested residences between 2017 and 2021. These populations developed different resistance levels towards several insecticides, especially fipronil and deltamethrin (Hu et al., 2020). A laboratory strain (EHI) that had been reared in the laboratory without any insecticide exposure for more than 40 yr (Chai and Lee, 2010), was used for comparison. All populations were maintained in round polyethylene containers (24 cm diameter × 32 cm height) under laboratory conditions of 25 ± 1°C, 50 ± 5% RH, and a photoperiod of 12:12 h light: dark. All populations were reared with corrugated cardboard harborage, dog food (RT-Mart chicken flavor dog food, Hsinchu, Taiwan), and water ad libitum.

Chemicals This study used technical-grade fipronil (98%, Toronto Research Chemicals, Inc., North York, Canada), imidacloprid (94%, Tagros Chemicals India Ltd., Chennai, India), and indoxacarb (90%, Shangdong Jingbo Agrochemicals Technology Co. Ltd., Shangdong, China). All chemicals were dissolved in absolute acetone (Union Chemical Works Ltd., Hsinchu, Taiwan) as stock solutions and stored at 4°C until use.

Bait selection The resistance development toward gel baits was evaluated with three field populations (BZ, CR, and WF) and three commercial gel baits. These baits were Jin-Li-Hai Ultra Max (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).

Before the experiment, the cockroaches were starved and provided with only water for 48 hours. A group of males and non-gravid females was introduced into a polyethylene container (36 × 28 × 12 cm) with harborage, water, and gel bait ad libitum. Treated cockroaches were exposed to gel baits without any alternative food for 24-48 hours, and the survival rate of treated cockroaches was recorded. Those cockroaches that survived post-treatment were transferred into a clean container and reared with dog food and water for breeding to the next generation. This selection process was repeated from the parental generation (F0) to the third generation (F_3).

Topical bioassay The heritability of resistance was evaluated with three field populations (BZ, CR, and WF). Each parental and selected generation of three populations was tested with a topical bioassay to generate LD_{50} and the slope of probit regression toward the corresponding insecticide used in bait selection.

Adult males of *B. germanica* were tested in this experiment. Series of diluted doses of fipronil (ranging from 0.5×10^{-3} to 2.5×10^{-1} mg mL^{-1}), imidacloprid (ranging from 1.265 to 2.016×10^{1} mg mL^{-1}), and indoxacarb (ranging from 1.5×10^{-1} to 8.445 mg mL^{-1}) were designed and used to generate 10-90% cockroach mortality. Before the experiment, the cockroaches were starved and provided with only water for 24 hours. After ice anesthesia, ten cockroaches were treated with insecticide. One microliter of diluted insecticide was applied to the cockroach's first and second abdominal sternites with a handled micro applicator (Burkard Scientific Ltd., Middlesex, United Kingdom). Treated cockroaches were maintained with dried dog food and water and the mortality of cockroaches was recorded at 48 h post-treatment. Each dose in the experiment was replicated three times. Ten males of each population were treated with acetone only for control.

Statistical analysis The mortality data of topical bioassay was calculated with probit analysis to estimate the values of LD_{50} and LD_{95}. The probit analysis was performed using R version 4.2.1. By dividing the LD_{50} values of the field populations with the corresponding susceptible strain (EHI) according to Chai and Lee (2010), the resistance ratio (RR_{50}) was calculated and further classified into five categories according to Lee and Lee (2004): ≤1 (absence of resistance); >1 to ≤5 (low resistance); >5 to ≤10 (moderate resistance); >10 to ≤50 (high resistance); and >50 (very high resistance).

To assess the risk of insecticide selection on resistance development, realized heritability (h^2) was estimated by using the method described by Tabashnik (1992) as $h^2 = R/S$ (R, the response to selection; S, the selection differential). Response to selection (R) was estimated as follows:

$$R = [\log(\text{final LD}_{50}) - \log(\text{initial LD}_{50})]/n$$

The final LD_{50} is the LD_{50} of offspring after n generations of selection. The initial LD_{50} is the LD_{50} of the parental generation (F_0) before bait selections, and n is the number of generations selected. The selection differential (S) was estimated as follows:

$$S = i \times \sigma\text{p}$$

i is the intensity of selection, and σp is the phenotypic standard deviation. The intensity of selection (i) is estimated as follows:

$$i = 1.583 - 0.0193336 + 0.0000428p^2 + 3.65194/p$$

p is the mean percentage of surviving rate (Tabashnik and McGaughey, 1994). The phenotypic standard deviation (σp) is estimated as follows:

$$\sigma\text{p} = [1/2(\text{mean slope})]^{-1}$$

The mean slope is the average slope of the probit regression lines from the parental generation before selection to the offspring after n generations of selection. The value of h^2 is determined by R and S. For a similar value of S, the population with lower h2 means a similar LD50 between the initial and final generation, which indicates lower resistance development.

With the values of response to selection (R), the number of generations required for a 10-fold increment of LD_{50} (G) can be estimated as the reciprocal of R:

$$G = R^{-1} = (h^2 S)^{-1}$$

RESULTS AND DISCUSSION

With the comparison of the values of RR_{50}, the resistance levels of parental generations (F_0) of BZ, CR, and WF were dissimilar from low to high for fipronil (1.4—19.4, Table 1), low for imidacloprid (2.1—2.6, Table 1) and low to moderate for indoxacarb (1.1—6.0, Table 1). Significant fipronil resistance was observed in BZ and WF, especially BZ with high RR_{50} (19.4, Table 1). The insecticide resistance observed in BZ and WF involved metabolic resistance with the elevated cytochrome P450 monooxygenase and esterase (Hu et al., 2020; 2021). Significant indoxacarb resistance was also observed in BZ and WF (Table 1), indicating a potential cross-resistance between fipronil and indoxacarb. We suggested that the potential cross-resistance is associated with metabolic resistance, as reported by Liang et al. (2017). CR exhibited low resistance toward three insecticides, whereas a significant RR_{50} was observed for imidacloprid (Table 1). BZ and WF also exhibited significant but low RR_{50} for imidacloprid (Table 1).

With the selection pressure from commercial baits, the offsprings of three populations exhibited distinct responses of insecticide resistance based on RR_{50}, realized heritability (h^2), and number of generations required for a 10-fold increment in LD_{50} (G). In fipronil selection, RR_{50} for fipronil in Fip. BZ remained high throughout generations, while the development of resistance was the slowest with h^2 and G among three selected strains (RR_{50} = 19.4—25.8, h^2 = 0.14, G = 24.4, Table 2). RR_{50} of Fip. WF showed rapid growth of resistance towards fipronil in a moderate-resistance background with the highest value of h^2 (RR_{50} = 8.9—18.6, h^2 = 0.40, G = 6.2, Table 2). Increasing RR_{50} was also observed in Fip. CR with lower h^2 compared to Fip. WF, whereas a 10-fold increase in LD_{50} could occur within approximately five generations (RR_{50} = 1.4—5.0, h^2 = 0.18, G = 5.5, Table 2). Rapid growth of fipronil resistance was observed in Fip. CR and Fip. WF. The development of fipronil-resistance in Fip. BZ was slow (Table 2), indicating that the resistance of fipronil in *B. germanica* may have reached an upper limit, as supported by the observations from González-Morales et al. (2022). in RR_{50} was observed under the treatment of imidacloprid-containing bait in selected strains. In Imi. BZ, RR_{50} elevated with a nonsignificant increment of LD_{50} (RR_{50} = 2.5—3.7, h^2 = 0.25, G = 16.9, Table 2). With a significantly increased value of LD_{50} and the highest h^2, RR_{50} of Imi. WF rapidly elevated (RR_{50} = 2.6—5.6, h^2 = 0.31, G = 9.1, Table 2). Compared to RR_{50} and h^2, resistance development was

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Table 1. Toxicity of fipronil, imidacloprid and indoxacarb against unselected field populations (F_0) of *B. germanica* from Taiwan at 48 h post-treatment

Insecticide	Strain	N	LD_{50} (95% FL) (μg/ g)	Slope ± SE	χ^2 (df)	[1]RR_{50}
Fipronil	BZ	270	1.94 (1.73 − 2.17)	4.554 ± 0.570	5.494 (4)	*19.4
	CR	360	0.14 (0.10− 0.18)	2.995 ± 0.278	8.096 (4)	1.4
	WF	330	0.89 (0.76− 1.01)	3.679 ± 0.377	4.271 (7)	*8.9
Imidacloprid	BZ	180	62.01 (37.27− 98.86)	4.397 ± 0.675	6.380 (3)	*2.5
	CR	270	51.91 (44.51 − 59.61)	5.464 ± 0.715	0.279 (5)	*2.1
	WF	240	66.37 (55.86− 76.71)	3.239 ± 0.445	1.354 (4)	*2.6
Indoxacarb	BZ	180	35.70 (27.59− 46.91)	2.166 ± 0.317	2.730 (3)	*6.0
	CR	240	6.80 (4.71− 8.97)	1.777 ± 0.259	1.960 (5)	1.1
	WF	270	21.20 (16.43 − 26.94)	1.992 ± 0.246	6.336 (6)	*3.5

[1]The values of resistance ratio of LD_{50} (RR_{50}) were calculated by dividing LD_{50} values of the field populations with LD_{50} values of the susceptible strain (EHI). Asterisks indicate the significant difference of insecticide resistance for field population compares to EHI based on non-overlap of 95% FL.

Table 2. Realized heritability (h^2) and number of generations required for a 10-fold increment in LD_{50} (*G*) for resistance towards fipronil, imidacloprid and indoxacarb in different selected strains of *B. germanica*

Estimated mean response per generation					Estimated selection di per generatioı	
Initial LD_{50} / g) (95% FL)	Initial RR_{50}	Final LD_{50} (µg/ g) (95% FL)	[3]Final RR_{50}	*R*	*p*	Mean slope
1.94 1.73−2.17)	19.4	2.58 (2.15−3.49)	25.8	0.041	31.9	3.73
0.14 0.10−0.18)	1.4	0.49 (0.38−0.62)	*5.0	0.181	3.1	2.23
0.89 0.76−1.01)	8.9	1.87 (1.66−2.07)	*18.6	0.161	20.0	3.47
62.01 7.27−98.86)	2.5	93.16 (85.03−102.21)	3.7	0.059	30.9	4.84
51.91 4.51−59.61)	2.1	63.10 (52.59−74.08)	2.5	0.028	25.1	3.71
66.37 5.86−76.71)	2.6	141.45 (121.44−172.23)	*5.6	0.110	24.9	3.62
35.70 7.59−46.91)	6.0	41.20 (31.17−60.92)	6.9	0.031	11.7	1.86
6.80 4.71−8.97)	1.1	14.25 (11.33−17.39)	*2.4	0.161	3.3	2.41
21.20 6.43−26.94)	3.5	26.44 (22.15−32.16)	4.4	0.048	3.6	2.61

[1]Strains selected by corresponding insecticide. Fip, fipronil; Imi, imidacloprid; Ind

noted in Ind. CR exhibited low resistance in the parental generation (F_0) (Table 2). This result was similar to the observations of indoxacarb bait performance by Hu et al. (2020). Indoxacarb baits demonstrated high efficacy, with high mortality observed across all field populations. This effectiveness may be attributed to the infrequent use of indoxacarb in Taiwan. As of 2022, commercial baits containing indoxacarb had not yet been registered to manage *B. germanica* in Taiwan (Hu et al., 2020).

In summary, we demonstrated the risk of insecticide resistance to fipronil-, imidacloprid- and indoxacarb-containing baits against field-resistant *B. germanica*. With the comparison of realized heritability (h^2) and number of generations required for a 10-fold increment in LD_{50} (G), we observed distinct patterns in the development of insecticide resistance, which varied depending on the resistance background. However, the resistant status is essential in determining the performance of fipronil- and imidacloprid-containing bait. Indoxacarb baits could be an alternative to manage the German cockroach populations and ensure satisfactory management against resistant German cockroaches in Taiwan.

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