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RESISTANCE RISK ASSESSMENT IN FIELD-COLLECTED STRAINS OF THE GERMAN COCKROACH (DICTYOPTERA: BLATTELLIDAE) TO FIPRONIL AND INDOXACARB BAITS

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Abstract The German cockroach, *Blattella germanica* (L.) is an important urban insect pest world-wide. Heavy reliance and frequent usage of residual insecticides have led to the development of insecticide resistance in the German cockroach. Bait formulations containing fipronil and indoxacarb are excellent resistance management tools against insecticide-resistant cockroaches. Despite that they have been used for nearly a decade, there are limited studies on the quantitative assessment of the development of fipronil and indoxacarb resistance in *B. germanica*. This study was carried out to assess the risk of resistance development towards fipronil and indoxacarb baits in three field-collected German cockroach populations (Boat Quay, Cavenagh and Ghimmoh strains) from Singapore, based on laboratory bait selection. These strains were each divided into two groups, one group selected with fipronil bait, while the other one selected with indoxacarb baits. After continuous selection for 7 generations, there were 9–11.2 fold increase in the LD_{50} of fipronil, and 24.1–68.7 fold increase in LD_{50} of indoxacarb, when compared to their parental generations. The realized heritability (h^2) of fipronil resistance ranged from 0.336 – 0.600, whereas indoxacarb resistance was in range of 0.197–0.475. The estimated slopes and h^2 were not correlated and all the possible combinations of these estimates provided prediction that it will take between 3.2 and 21.3 generations, and between 1.5 and 16.9 generations to achieve 10-fold increase in resistance to fipronil and indoxacarb, respectively, under a selection pressure of 50–90% mortality. This is the first study on resistance risk assessment in the German cockroach to fipronil and indoxacarb baits using multiple field strains. The outcome of this study provides useful information to the pest management industry towards preventing or delaying insecticide resistance development to baits in *B. germanica*.

Key words Realized heritability, resistance development, selection, insecticide resistance.

INTRODUCTION

The German cockroach, *Blattella germanica* (L.), is one of the most important cosmopolitan urban insect pest (Rust et al., 1995; Lee, 2007). Frequent usage and heavy reliance on insecticides have led to the development of insecticide resistance in this species (Cochran, 1995; Hemingway et al., 1995; Lee et al., 1996; Wei et al., 2001; Chai and Lee, 2010). Fipronil and indoxacarb are commonly used toxicants in cockroach baits. Earlier reports have recorded the occurrence of physiological-based resistance of fipronil (Holbrook et al., 2003; Kristensen et al., 2005; Gondhalekar and Scharf, 2012; Ang et al., 2013) and indoxcarb (Gondhalekar et al., 2011; Ang et al., 2014) in the German cockroach.

Assessing resistance risk is an important component of integrated pest management (IPM) and resistance management program. Resistance risk can be assessed via artificial selection in the laboratory (NRC, 1986) and subsequent quantitative genetic analyses of the data. By considering that resistant/ susceptive to an insecticide is a threshold trait, the realized heritability is estimated based on actual inter-generational responses to selection to predict the speed and potential amount genetic changes in future generations (Firko and Hayes, 1990; Hartl, 1988). Tabashinik (1992) suggested that brief selection experiments (4–6 generations) may effectively detect the potential for resistance development. Too short selection experiment might produce an unreliable estimated heritability because of the short-term random changes in insect population and experimental errors (Firko and Hayes, 1990).

In this study, we assessed the resistance risk development of fipronil and indoxacarb using laboratory bait selection method against three strains of field-collected populations of *B. germanica* from Singapore, and subsequently predicted the risk of resistance development of these two toxicants with the estimated realized heritability.

MATERIALS AND METHODS

Cockroach strains. The cockroaches used were the Boat Quay, Cavenagh Road and Ghimmoh Road strains from Singapore. The resistance profiles of these strains were previously described in Chai and Lee (2010) and Ang et al. (2013). A laboratory susceptible strain (EHI) was used as comparison. All cockroaches were reared under laboratory conditions of 26 ± 1ºC, 60 ± 5 % RH and photoperiod (12:12, L:D) with food and water provided ad libitum.

Chemicals. Technical grade insecticides fipronil (PestAnal, Sigma-Aldrich Laborachemikalien GmBh, Munich, Germany) and indoxacarb (Sigma-Aldrich Sdn. Bhd., Kuala Lumpur, Malaysia) were used in topical assay. The Goliath® gel bait (Bayer Environmental Sciences, Singapore) containing 0.05% fipronil and Advion® gel (Syngenta Crop Protection, Malaysia) containing 0.6% indoxacarb were used for bait selection and evaluation.

Selection for resistance. The method used for the selection experiment was identical to that described in Ang et al. (2013). Groups of nymphs (approximately ten thousand individuals each) from the three cockroach strains was selected with fipronil bait, while the other group was treated with indoxacarb bait. The EHI susceptible strain was reared without any exposure to the baits as control (unselected). Approximately 3 g of gel bait was provided for 24 – 48 hour, without the presence of alternative food. After the treatment, the bait was removed, and dried dog food and water were introduced to the survivors. Offspring of the survivors were tested for susceptibility to the insecticides using topical assays. Cockroaches were selected based on the described procedure for up to the 7th generation.

Topical bioassays. One to 3 weeks old adult males were topically treated with a series of concentrations of fipronil diluted in acetone using a microapplicator (Burkard Scientific Ltd., Middlesex, United Kingdom). The control set was treated with one µl of acetone. All treated cockroaches were transferred into a clean petri dish (90 mm diam. x 15 mm height) provided with dog food and a wet cotton ball. Mortality was scored at 48 hours post-treatment.

Data analysis. Data were pooled and subjected to probit analysis using POLO-PC (LeOra, 1997). Resistance ratio (RR_{50}) was determined by comparing the LD_{50} of selected population with those of the susceptible one.

Estimation of realized heritability. The heritabilities of resistance to indoxacarb and fipronil in *B. germanica* were estimated as realized heritability (h^2) based on a threshold trait analysis method up to 7th selected generation using the equation:

$$h^2 = R/S$$

where *R* is the response to selection and *S* represents the selection differential (Tabashnik, 1992; Falconer, 1989). The h^2 values were calculated separately for three selected populations.

The *R* value, the difference in mean phenotype (insecticide tolerance level) between progeny of selected parents and the whole parental generation prior to selection, was calculated based on a formula:

$$R = \frac{\log(\text{final } LD_{50}) - \log(\text{initial } LD_{50})}{n}$$

where the final LD_{50} is the lethal dose of progeny of the selected cockroach population at 50% mortality after *n* generations of selection, the initial LD_{50} is the lethal dose of parental generation at 50% mortality and *R* is the average selection response of single generation.

The *S* value, the difference in mean phenotype (insecticide tolerance level) between the selected parents and the whole parental generation, was estimated as

$$S = i\sigma_p$$

where *i* is the intensity of selection and σ_p σ_p is the phenotypic standard deviation. The *i* value was estimated from *p*, the percentage of surviving individual after selection by using Falconer and Mackay (1996), based on the properties of the normal distribution. The phenotypic standard deviation (σ_p) was estimated as the reciprocal of the mean of the estimated slopes of the probit regression lines from the parental generation (initial slope) and the progeny after *n* generations of selection (final slope):

$$\sigma_p = \frac{1}{{}^1\!/_2\,(initial\ slope + final\ slope)}$$

Projected rates of resistance development. The future response of selection (*R*) can be estimated as the multiplication of heritability (h^2) and selection differential (*S*),

$$R = h^2 S$$

Using the response of selection of the three laboratory-selected cockroach populations, the number of generations that required for a 10-fold increasing in LD_{50}, *G*, is the reciprocal of *R*, can be predicted with different selection pressure (*S*), phenotypic standard deviation (σ_p) and heritability (h^2).

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

RESULTS

After 7 generations of selection, there were increase of 9–11.2 folds in LD_{50} of fipronil (Figure 1) and 24.1–68.7 folds in LD_{50} indoxacarb (Figure 2), compared with the parental generation. The selection response *R* and selection differential *S* of fipronil resistance in the three selected populations were estimated to be 0.136–0.150 and 0.25–0.42, respectively. The estimated realized heritability (h^2) of fipronil resistance ranged from 0.336 to 0.600 (Table 1). On the other hand, with *R* of 0.197–0.262, and *S* of 0.53–1.12, indoxacarb resistance was found to develop in the three selected populations with h^2 of 0.197–0.475 (Table 1). There was no apparent correlation between the mean slopes and h^2 among the strains for both fipronil and indoxacarb (Table 1).

Table 1. Estimation of response to selection (*R*) and selection differential (*S*) of fipronil- and indoxacarb-selected population of the German cockroach.

Strain	Estimation of response to selection			Estimation of selection differential			h^2
	Initial LD_{50} (95% FL)	Final LD_{50} (95% FL)	*R*	Mean slope	σ_p	*S*	
Fipronil-selected							
Boat Quay	0.07 (0.05–0.09)	0.75 (0.62–0.86)	0.147	5.71	0.18	0.25	0.600
Cavenagh Road	0.15 (0.09–0.27)	1.69 (1.28–2.00)	0.150	3.35	0.30	0.42	0.360
Ghimmoh Road	0.11 (0.09–0.13)	0.99 (0.69–1.22)	0.136	3.45	0.29	0.41	0.336
Indoxacarb-selected							
Boat Quay	21.85 (15.24–30.34)	760.86 (534.80–1031.96)	0.220	1.26	0.80	1.12	0.197
Cavenagh Road	41.24 (35.17–50.03)	993.20 (809.31–1251.67)	0.197	2.67	0.38	0.53	0.376
Ghimmoh Road	10.47 (7.97–14.91)	719.03 (586.04–859.48)	0.262	2.54	0.39	0.55	0.475

The projected rates of resistance development for fipronil and indoxacarb were estimated based on realized heritability (h^2) and selection differential (*S*). Figure 3 shows the effect of three different realized heritabilities (based on current study) on the number of generations required for a 10-fold increase in LD_{50} of fipronil at different selection intensities (*i*). Figure 4 shows effect of three different realized heritabilities (based on current study) on the number of generations required for a 10-fold increase in LD_{50} of indoxacarb at different selection intensities (*i*). It would take 1.5–16.9 generations for a 10-fold increase in LD_{50} of indoxacarb at different combination of slopes and estimated h^2 (Figure 4).

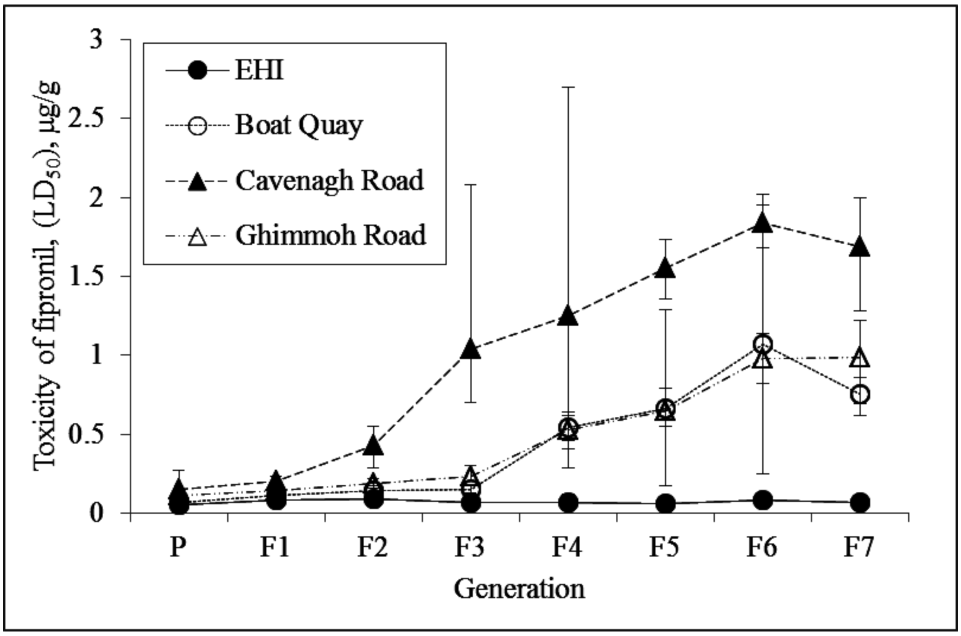

Figure 1. Development of fipronil toxicity in fipronil-selected *B. germanica* from parental to F7 generations (Results for parental to F5 generations had been reported in Ang et al. 2014). The error bars represent the range of the observed LD_{50}.

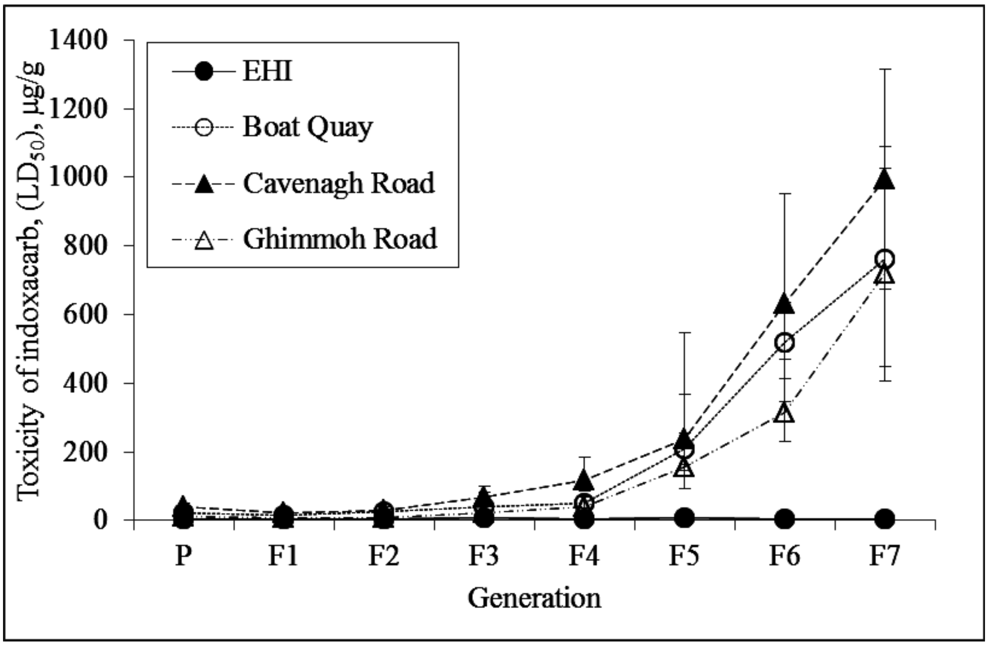

Figure 2. Development of indoxacarb toxicity in indoxacarb-selected *B. germanica* from parental to F7 generations (Results for parental to F5 generations had been reported in Ang et al. 2014). The error bars represent the range of the observed LD_{50}.

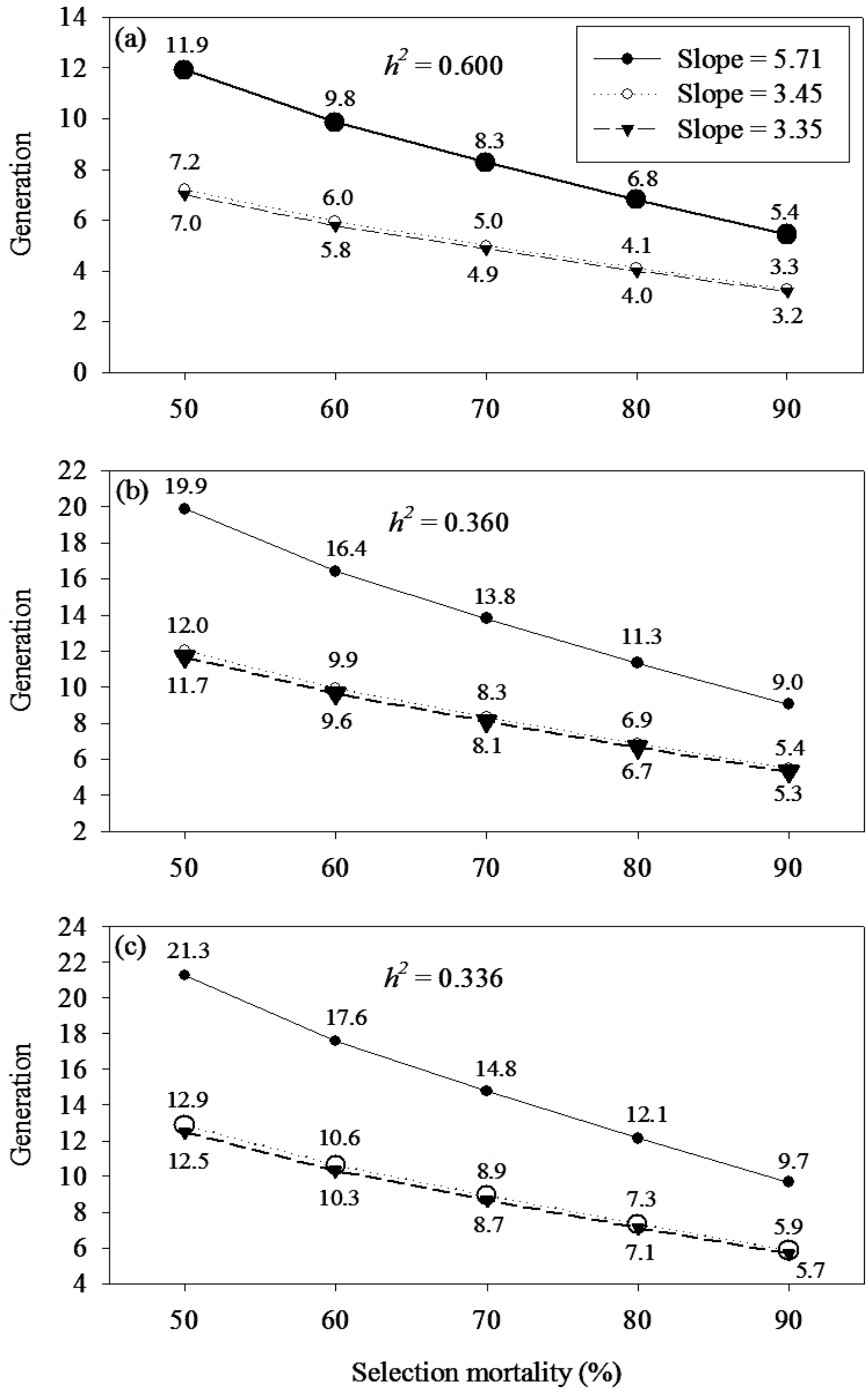

Figure 3. Effect of three different realized heritability [(a) $h^2 = 0.600$, (b) $h^2 = 0.360$, and (c) $h^2 = 0.336$] on the number of generations required for a 10-fold increase in LD_{50} of fipronil at three different slopes and different selection mortality (i). The three values of h^2 and slopes used were those obtained from the three populations. The lines with enlarged symbols are predictions based on the combination of estimated slopes and h^2 of each population.

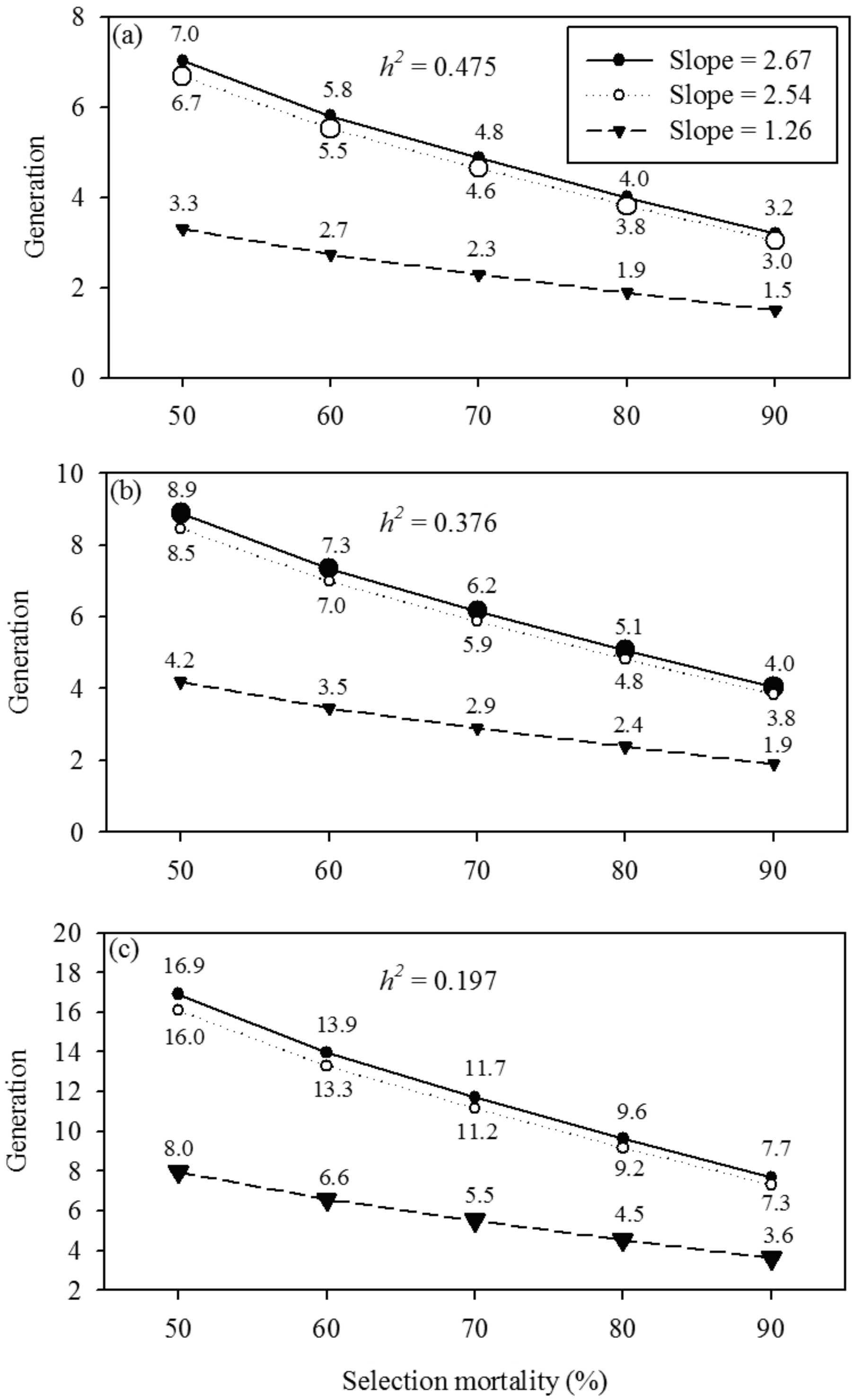

Figure 4. Effect of three different realized heritability [(a) $h^2 = 0.475$, (b) $h^2 = 0.376$, and (c) $h^2 = 0.197$] on the number of generations required for a 10-folds increase in LD_{50} of indoxacarb at three different slopes and different selection mortality (*i*). The three values of h^2 and slopes used were those obtained from the three populations. The lines with enlarged symbols are predictions based on the combination of estimated slopes and h^2 of each population.

DISCUSSION

We predicted that fipronil and indoxacarb resistance would increase at the rate of 34 – 60 % and 20 – 48 % of the selection differential, respectively. In this study, the range of estimated h^2 of fipronil resistance in the three fipronil-selected cockroach strains was 0.336 to 0.600, which was higher than that of rice stem borer, *Chilo suppressalis* (Walker) to fipronil (h^2 = 0.213) (Huang et al., 2010). In the case of indoxacarb resistance, the estimated h^2 in the selected cockroach populations was 0.197 to 0.475, which was higher than that of tobacco budworm, *Heliothis virescens* (Fabrius) (h^2 = 0.03) (Sayyed et al., 2008), but was comparable with that of cotton bollworm, *Helicoverpa armigera* (Hubner) (h^2 = 0.45) (Ghodki et al., 2009).

We used three strains to diversify the responses from different cockroach populations with varying resistance allele frequency. These strains were previously reported to carry 302Ser resistance allele (*Rdl* mutation), responsible for fipronil resistance at the varying frequencies (Boat Quay = 17.5%, Cavenagh Road = 35.0% and Ghimmoh Road = 12.5%) (Ang et al. 2013). No relationship between the initial frequency of resistance allele and the realized heritability was detected. However, Tabashnik (1992) reported that the initial resistance allele frequency did play significant role in estimating h^2 in first few generations of selection. This could likely due to the existence of some addition variations such as modifier loci (Firko and Hayes, 1990). The effects of this variation are normally diverse among different cockroach strains. Hence, it is important to use multiple strains to determine selection responses with different genetic backgrounds.

In this study, we used realized heritability (h^2 = 0.336–0.600 for fipronil resistance, h^2 = 0.197–0.475 for indoxcarb resistance) to predict the rate of resistance development with all possible combinations of different probit slopes based on different selection intensities (Figure 3 and 4). We found that the 10-folds increase in LD_{50} of fipronil can happen within only three generations, while it can take up to 21 generations if the selection pressure is low. Similar situation was observed in the selection using indoxacarb bait.

There are some limitations and biases when accounting artificial selection experiment for insecticide resistance traits. Rosenheim (1991) described three processes that can result in over- and underestimating of heritability; the difference in receiving doses by different individuals, sublethal effects of insecticide after exposure (reduced fitness components) and unequal selection of males and females. If there is negative pleiotropic effect between resistance and fitness components, insecticide selection shall result in reduced fitness and this may lead to underestimation in resistance response. Our earlier study demonstrated that these strains showed absence of fitness penalty (Ang et al., 2011). In this study, we reduced the bias by only using the immature stages to prevent premating selection of reproductive individuals. If artificial selection was done on the adults of both sexes which mating may occur before the selection, this may result in the *S* value become smaller for males compared to females due to the possible difference of tolerance distribution of both sexes (Rosenheim, 1991).

This study demonstrated for the resistance risk assessment of fipronil and indoxacarb gel baits on German cockroaches for the first time. The information obtained from this study will assist in developing an effective IPM program against this species, and to prevent the development of physiological-based resistance towards these two toxicants.

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