Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

INDOXACARB RESISTANCE IN THE GERMAN COCKROACH AFTER BAIT SELECTION

[1,2]LING-HUI ANG, [3]NAZNI WASI AHMAD, AND [1]CHOW-YANG LEE
[1]Urban Entomology Laboratory, Vector Control Research Unit,
School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia
[2]WellTech Healthcare Co. Ltd., 183 Regent House Building Fl. 19,
Thanon Ratchadamri Lumpini, Pathumwan, Bangkok, Thailand
[3]Division of Medical Entomology, Institute for Medical Research,
Jalan Pahang, 50588 Kuala Lumpur, Malaysia

Abstract In this study, we examined the resistance status of six field-collected strains from Singapore to eight insecticides, as well as the resistance of the parental through F_5 generations after they were selected with indoxacarb bait. All strains showed no resistance to acetamiprid (0.7–1.9x), imidacloprid (1.6–2.5x), chlorantraniliprole (2.3–4.0x), and bendiocarb (2.1–2.7x); low levels of resistance to indoxacarb (2.5–9.9x) and chlorpyrifos (2.6–8.7x); and moderate levels of resistance to DDT (>2.6x) and deltamethrin (5.8–55.6x). Very low resistance levels were detected in the indoxacarb bait assay test. Cross-resistance between deltamethrin and indoxacarb was found where LD_{50} and RR_{50} values for deltamethrin and indoxacarb were positively correlated ($P < 0.01$). Subsequent selection with indoxacarb bait on three strains significantly ($P < 0.05$) increased indoxacarb resistance (up to >30x), but it reduced deltamethrin resistance.
Key words Cross-resistance, *Blattella germanica*, pyrethroid.

INTRODUCTION

Heavy reliance on insecticides and high frequency of application have led to the development of insecticide resistance in the German cockroach, *Blattella germanica*. Field populations of *B. germanica* have developed multiple insecticide resistance mechanisms such as reduced cuticular penetration, increased detoxification rate and target site insensitivity. The German cockroach has developed resistance to at least 47 different chemicals, including to novel insecticides such as fipronil (Kristensen et al., 2005; Ang et al., 2013) and indoxacarb (Chai and Lee, 2010).

Indoxacarb is the first commercialized pyrazoline-like insecticide that acts by blocking the independent site of the sodium channel (McCann et al., 2001). It is readily bioactivated by esterase/amidase type enzymes to yield the potent insecticidally active bio-product *N*-decarbomethoxylated metabolite (DCJW), which results in pseudoparalysis and subsequent death of poisoned insects (Wing et al. 1998; Wing et al. 2000; Wing et al., 2005). The mode of action of indoxacarb and its metabolite are novel and entirely distinct from that of pyrethroid.

Chai and Lee (2010) reported that several field-collected German cockroach populations from Singapore were resistant to indoxacarb, even though these cockroach populations were never exposed to this insecticide. We suspect that this phenomenon was due to cross-resistance from pyrethroid. Several insect pests have shown cross-resistance between pyrethroid and indoxacarb due to elevated monooxgenase and esterase (Ahmad and Hollingworth, 2004; Shono et al., 2004;

Ahmad and Arif, 2009; Nehare et al., 2010). However, information about the indoxacarb resistance mechanism and the potential for cross-resistance between pyrethroid and indoxacarb in *B. germanica* are limited.

This study reports the resistance status of six field-collected strains of the German cockroach from Singapore to indoxacarb and other commonly used insecticides. In addition, three strains were selected and subjected to insecticide selection using indoxacarb bait.

MATERIALS AND METHODS

Six field-collected strains of German cockroach were used in this study: B1 Tampines Central, Beach Road, Boat Quay, Victoria Street, Cavenagh Road, and Ghimmoh Road. A laboratory insecticide-susceptible reference strain that originated at the Environmental Health Institute (EHI), Singapore was used for comparison. Technical grade deltamethrin, chlorpyrifos, imidacloprid, DDT and bendiocarb, indoxacarb, chlorantraniliprole, and acetamiprid diluted in analytical grade acetone were used.

Ten adult males aged 1–3 weeks were immobilized with CO_2 prior to topical application. One µl of insecticide solution was placed on the first segment of the cockroach abdominal sternites using a microapplicator (Burkard Scientific Ltd., Middlesex, UK). Treated cockroaches were transferred into a clean Petri dish supplied with dog food and a wet cotton ball. Mortality of the cockroaches was scored 48-h after treatment. A total of 3–6 doses resulting in > 0% to <100% cockroach mortality, were evaluated for each insecticide and the experiment was replicated 3 – 5 times.

Advion® (0.6% indoxacarb) cockroach gel bait was used for the bait assay against the susceptible and field-collected strains. Ten adult males were released into the test arena that was provisioned with harborage and water and acclimatized for 24-h before the bait placement. Dead or unhealthy individuals were replaced immediately before the bait assay. Dog food was provided ad libitum and placed at one corner of the arena, while 0.1 g gel bait was placed at the other corner. Mortality of the cockroaches was scored every 1–3 h until all cockroaches were dead. Each bait assay was replicated four times.

Based on the topical bioassay results, three strains (Cavenagh Road, Ghimmoh Road, and Boat Quay) were chosen and subjected to bait selection. Approximately 3 g of indoxacarb bait were introduced to the cockroaches in the rearing tank without an alternative food source. After 24–48 h of exposure when approximately 80% of the test cockroaches were dead, the dead and moribund cockroaches, and the bait were removed from the rearing tank. The remaining survivors were provided with dry dog food and reared under laboratory conditions. Adult male offspring (aged 1–3 weeks) of selected generations of the three strains were evaluated with topical and bait assays to determine the effect of bait selection on the susceptibility of the cockroach populations to indoxacarb.

All mortality data were subjected to probit analysis. Resistance ratio (RR) was calculated by dividing the LD of field-collected strains by the corresponding LD of the susceptible strain. The RR was classified into five categories according to Lee and Lee (2004) and Chai and Lee (2010): <1, absence of resistance; 1 to 5, low resistance; 5 to 10, moderate resistance; 10 to 50, high resistance; and >50, very high resistance. The synergism ratio (SR) was calculated by dividing the LD of deltamethrin alone by the LD of deltamethrin + synergist for the same strain. Comparisons of LD and LT values among cockroach strains were based on the overlap of their respective 95% fiducial limits (FLs). Relationships between all tested insecticides were determined using pairwise correlation analysis of LD and RR values.

RESULTS

Based on the toxicity results of the EHI strain, deltamethrin was the most toxic insecticide, whereas acetamiprid was the least toxic. The Cavenagh Road strain showed the highest resistance level among the field-collected strains. The results showed that all strains were susceptible to acetamiprid, imidacloprid and chlorantraniliprole. Deltamethrin resistance levels ranged from 5.8 to 55.6-fold, and DDT resistance levels were >2.6-fold. Low to moderate indoxacarb resistance was found in several strains. The Boat Quay, Cavenagh Road, and Ghimmoh Road strains showed low to moderate chlorpyrifos resistance. In contrast, no chlorpyrifos resistance was detected in the B1 Tampines Central, Beach Road, and Victoria Street strains. Low resistance to bendiocarb was also recorded in the studied strains. Out of the six field-collected strains tested on indoxacarb bait, only two strains exhibited no significant difference in LT_{50} values compared to that of EHI susceptible strain. The Cavenagh Road and Ghimmoh Road strains showed significantly higher LT_{50} values than the EHI susceptible strain.

Pairwise correlations of LD_{50} and RR_{50} values among field-collected strains for all insecticides tested revealed no correlation between all insecticides and the cockroach strains, except for a positive significant correlation ($P < 0.01$, $R = 0.973$ for LD_{50}, $R = 0.972$ for RR_{50}) between deltamethrin and indoxacarb.

Boat Quay, Cavenagh Road, and Ghimmoh Road exhibited increased indoxacarb resistance after selection. These strains showed reduced resistance against three other conventional insecticides (deltamethrin, bendiocarb, and chlorpyrifos). The Boat Quay strain exhibited the highest LT_{50} at F_5 generation. This value was significantly different ($P < 0.05$) from that of the parental generation. The F_5 generations of the Cavenagh Road and Ghimmoh Road strains showed significantly higher ($P < 0.05$) LT_{50} values compared to that of the EHI susceptible strain (Figure 1).

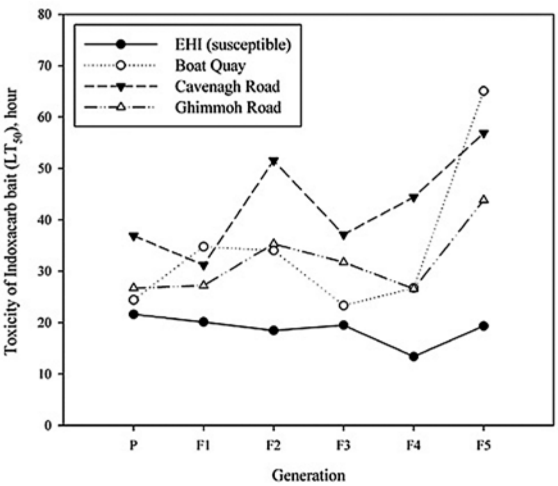

Figure 1. LT_{50} of all generations (from parental to F_5) of three indoxacarb-selected cockroach strains determined using bait assay.

DISCUSSION

The topical bioassay revealed a low level of indoxacarb resistance in the cockroach strains. Indoxacarb resistance was possibly caused through cross-resistance from deltamethrin and was shown in the correlation analysis. Indoxacarb in bait formulation, was never used against these populations of the German cockroaches in the field. The bait assay revealed that our cockroach strains had a significantly lower resistance to indoxacarb (i.e., oral ingestion) compared to topical application (Gondhalekar et al.

2011; Gondhalekar et al. 2013). This likely is because the midgut cells of cockroaches contain a high concentration of esterase/amidase-like enzymes, which are responsible for indoxacarb bioactivation (Wing et al., 1998; Wing et al., 2005). Reierson (1995) reported that a single adult German cockroach can consume at least 1 mg of food in a single meal. The indoxacarb bait formulation that we used contains 0.6% of active ingredient; thus, a cockroach will ingest ~6 µg of indoxacarb in 1 mg of bait. In addition, this compound exhibits poor cuticular penetration (Yu and McCord, 2007). The cross-resistance between members of pyrethroid class and indoxacarb, had been reported in other pest insects, such as the spotted bollworm *Earias vittella* (F) (Ahmad and Arif, 2009), obliquebanded leafroller *Choristoneura rosaceana* (Harris) (Ahmad and Hollingworth, 2004), and housefly *Musca domestica* (L) (Shono et al., 2004).

Deltamethrin and indoxacarb share a similar target site (i.e., the voltage-gated sodium channel) (Soderlund 2005). However, the differences in mode of action (Lapied et al., 2001; Narahashi, 2002; Yu, 2008) and binding sites in the channel (Wing et al., 2005) might explain the different resistance levels of deltamethrin (5.8–55.6x) and indoxacarb (2.5–9.9x).

After 5 generations of selection, no correlation was found between the susceptibility of the cockroaches to indoxacarb and those of deltamethrin. The deltamethrin resistance in the indoxacarb-selected populations was significantly reduced from that of the parental generations. The selection process using indoxacarb bait might have changed the resistance mechanisms in these strains that they became indoxacarb-specific. Deltamethrin may select for low indoxacarb resistance, however it is not possible vice-versa. Indoxacarb gel is still considered effective against these indoxacarb-selected cockroaches as all cockroaches died within 2 weeks after treatment.

In conclusion, cross-resistance is an important issue to consider, especially when a new insecticide with a similar target site is being introduced. This problem should not be underestimated because of its potential in shortening the longevity of the effectiveness of an insecticide. An understanding of the resistance mechanism and how resistance develops are essential to develop an effective resistance pest management strategy against urban insect pests.

REFERENCES CITED

Ang, L.H., W.A. Nazni, M.K. Kuah, A.C. Shu-Chien, and C.Y. Lee. 2013. Detection of the A302S Rdl mutation in fipronil bait-selected strains of the German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 106: 2167 – 2176.

Ahmad, M. and M. I. Arif. 2009. Resistance of Pakistani field populations of spotted bollworm *Earias vittella* (Lepidoptera: Noctuidae) to pyrethroid, organophosphorus and new chemical insecticides. Pest Manag. Sci. 65: 433-439.

Ahmad, M. and R. M. Hollingworth. 2004. Synergism of insecticides provides evidence of metabolic mechanisms of resistance in the obliquebanded leafroller *Choristoneura rosaceana* (Lepidoptera: Tortricidae). Pest Manag. Sci. 60: 465-473.

Chai, R. Y. and C. Y. Lee. 2010. Insecticide resistance profiles and synergism in field populations of the German cockroach (Dictyoptera: Blattellidae) from Singapore. J. Econ. Entomol. 103: 460-471.

Gondhalekar, A. D., C. W. Scherer, R. K. Saran, and M. E. Scharf. 2013. Implementation of an indoxacarb susceptibility monitoring program using field-collected German cockroach isolates from the United States. J. Econ. Entomol. 106: 945-953.

Gondhalekar, A. D., C. Song, and M. E. Scharf. 2011. Development of strategies for monitoring indoxacarb and gel bait susceptibility in the German cockroach (Blattodea: Blattellidae). Pest Manag. Sci. 67: 262-270.

Kristensen, M., K. K. Hansen, and K.-M. V. Jensen. 2005. Cross-resistance between dieldrin and fipronil in German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae). J. Econ. Entomol. 98: 1305-1310.

Lapied, B., F. Grolleau, and D. B. Sattelle. 2001. Indoxacarb, an oxodiazine insecticide, blocks insect neuronal sodium channels. Brit. J. Pharmacol. 132: 587-595.

Lee, L. C. and C. Y. Lee. 2004. Insecticide resistance profiles and possible underlying mechanisms in German cockroaches, *Blattella germanica* (Linnaeus) (Dictyoptera: Blattellidae) from Peninsular Malaysia. Med. Entomol. Zool. 55: 77-93.

Nehare, S., B. S. Ghodki, G. K. Lande, V. Pawade, and A. S. Thakare. 2010. Inheritance of resistance and cross resistance pattern in indoxacarb-resistant diamondback moth *Plutella xylostella* L. Entomol. Res. 40: 18-25.

Reierson, D. A. 1995. Baits for German cockroach control, pp. 231-265. *In* M. K. Rust, J. M. Owens and D. A. Reierson [eds.]. Understanding and controlling the German cockroach., Oxford University Press, New York.

Shono, T., L. Zhang, and J. G. Scott. 2004. Indoxacarb resistance in house fly, *Musca domestica*. Pestic. Biochem. Physiol. 80: 106-112.

Wing, K. D., M. E. Schnee, M. Sacher, and M. Connair. 1998. A novel oxadiazine insecticide is bioactivated in Lepidopteran larvae. Arch. Insect Biochem. Physiol. 37: 91-103.

Wing, K. D., J. T. Andaloro, S. F. McCann, and V. L. Salgado. 2005. Indoxacarb and the sodium channel blocker insecticides: chemistry, physiology, and biology in insects. Comprehensive Molec. Insect Sci. 6: 31-53.

Wing, K. D., M. Sacher, Y. Kagaya, Y. Tsurubuchi, L. Mulderig, M. Connair, and M. Schnee. 2000. Bioactivation and mode of action of the oxadiazine indoxacarb in insects. Crop Protec. 19: 537-545.

Yu, S. J., and E. J. McCord. 2007. Lack of cross-resistance to indoxacarb in insecticide-resistant *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Plutella xylostella* (Lepidoptera: Yponomeutidae). Pest Manag. Sci. 63: 63-67.