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STUDIES ON INSECTICIDE RESISTANCE IN AUSTRALIAN BED BUGS, *CIMEX* SPP. (HEMIPTERA: CIMICIDAE)

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Abstract To investigate insecticide resistance in modern field populations of bed bugs in Australia, a program to collect and screen field specimens was initiated. Thirty-five bed bug strains (a mixture that included both *C. lectularius* and *C. hemipterus*) were collected from across Australian and subsequently colonized for laboratory-based insecticide resistance testing. To screen for resistance, bed bugs were exposed topically to a discriminating dose of 2.5 g/L deltamethrin and, separately, 0.1 g/L of imidacloprid, applied at a rate of 1 µL/bug. Results indicated a broad spread in the frequency of resistance against deltamethrin in *C. lectularius*. Mortality was predominantly high with *C. lectularius* against imidacloprid at ≥95%, except for several recently collected strains that returned between 70%-85%, indicating that a degree of tolerance may be developing in select strains. Three of four *C. hemipterus* strains returned mortality values at or below 10%, with the remainder recording 75% mortality, thereby indicating pyrethroid resistance is similarly advanced in this species. Normal susceptibility (≥95% mortality) was otherwise evident in all four *C. hemipterus* strains to imidacloprid. These findings complement concurrent research, which indicates variable patterns of resistance mechanisms are present across the Australia and around the world. The results have profound implications for product manufacturers and registration authorities in ensuring that a product is as effective at controlling bed bugs in the field as the laboratory.

Key words *Cimex lectularius*, *Cimex hemipterus*, insecticide resistance

INTRODUCTION

Insecticide resistance is now considered as one of the key drivers of the global bed bug resurgence (Doggett et al., 2012; Romero et al., 2007) with resistance, particularly to the pyrethroid insecticides, well established in populations of both *C. lectularius* and *C. hemipterus* around the world (Adelman et al., 2011; Boase et al., 2006; Dang et al., 2015b; Dang et al., 2015c; Karunaratne et al., 2007; Kilpinen et al., 2011; Koganemaru et al., 2013; Moore and Miller, 2006; Myamba et al., 2002; Romero et al., 2007; Tawatsin et al., 2011; Yoon et al., 2008). In Australia, resistance was suspected by the mid-2000s following anecdotal reports from professional pest managers of poor insecticide performance (Doggett et al., 2012; Doggett and Russell, 2008) and, principally, mediocre efficacy with both pyrethroid and carbamate-based products when assessed by both topical and residual application under laboratory conditions (Lilly et al., 2009a, 2009b). Subsequently, full examination of dose-responses and resistance ratios between a susceptible and suspected-resistant strain via topical application confirmed extremely high resistance to deltamethrin and permethrin, along with intermediate resistance to bendiocarb (Lilly et al., 2015). No resistance to imidacloprid was detected at the time, although preliminary testing with a formulated product showed poor performance via residual application (Lilly and Doggett, unpublished data). Successive elucidation of *kdr*-type mutations determined that the majority of ‘modern’ strains possessed the haplotype B (L925I) target-site mutation, but that a smaller subset collected at or pre-2002, possessed either no mutation or a novel putative *kdr* mutation, haplotype I936F (Dang et al., 2015b). This suggested that *C. lectularius* populations across Australia were either rapidly evolving or new founders were introduced. Similarly, in *C. hemipterus*, four novel mutations on the voltage-gated sodium channel gene were discovered from colony and preserved specimens, of which two (M918I and L1014F) were deemed to be probable *kdr*-type mutations (Dang et al., 2015c). A contingent link of the above *kdr*-type mutations to expressed resistance was determined in seven strains (three Australian, four international) by exposure to a simplified 'mat-assay' designed for rapid and economical field use by professional pest managers (Dang et al., 2015a).

There still remains a gap in our present understanding as to how widespread resistance to pyrethroids is in recently collected field strains and whether there is evidence for any change in the frequency of resistance to pyrethroids and susceptibility to neonicotinoids. It was the aim of this study to screen field strain bed bugs collected from across Australia using a laboratory topical bioassay employing discriminating doses of the pyrethroid deltamethrin and the neonicotinoid imidacloprid. A secondary aim was to assess if resistance or susceptibility was changing over time by plotting mortality values against the collection date of each strain and analyzing the data for trends using linear regression.

MATERIALS AND METHODS

Thirty-one field strains of *C. lectularius* and four field strains *C. hemipterus* were derived from active bed bug infestations around Australia. After collection or receipt of field samples, all specimens were identified to species (Usinger, 1966), and select strains tested for *kdr*-type mutations (Dang et al., 2015a; Dang et al., 2015b; Dang et al., 2015c). Colonies were maintained at 25°C (±1°C) and 75% (±10%) RH with a photoperiod of 12:12 h (L:D). No insecticide selection was undertaken and all strains collected during or post-2012 were tested for their susceptibility within twelve months of their collection in order to eliminate any bias as a result of potential resistance reversion.

LD_{99} values from an insecticide-susceptible strain were based on data obtained with the 'Monheim' *C. lectularius* strain (Lilly et al., 2015), obtained from Bayer CropScience (Monheim, Germany) and which has been maintained under laboratory conditions since the 1960s. No equivalent insecticide-susceptible *C. hemipterus* strain exists, and data from the Monheim strain was regarded as indicative for *C. hemipterus* is selecting the same discriminating doses.

Discriminating Dose Bioassays

To determine topical resistance, a dose of 2.5 g/L deltamethrin in acetone applied at a rate of 1 µL/bug was set as the discriminating value and, for the neonicotinoids, a dose of 0.1 g/L of imidacloprid was chosen, also applied topically at 1 µL/bug. The deltamethrin dose was equivalent to approximately 10 times the dose of many formulated insecticides registered for the treatment of bed bugs, and was otherwise >500 times the LD_{99} of a *C. lectularius* susceptible strain. As no topical resistance to imidacloprid has ever been detected locally, the discriminating dose was selected based on it being approximately 4 times the LD_{99} of a *C. lectularius* susceptible strain. Controls received acetone only. Two replicates of 10 randomly selected adult bed bugs (mixed sex and age) per treatment plus controls were then affixed dorsally on double-sided tape to the lids of 500 mL plastic containers, and then 1 µL of solution was applied topically to the ventral side using a micro-applicator (Lilly et al., 2015). After exposure, the solution was allowed to dry for several minutes before the bugs were carefully removed with a fine pair of tweezers and placed in the base of the 500 mL container lined with ø90 mm Whatman number 1 qualitative filter papers. Mortality was recorded at 24 h and defined as the bugs exhibiting no response to gentle pressure from a probe or, in the case of moribund vestigial nerve twitching, the inability to right themselves upon being inverted onto their dorsal side.

RESULTS AND DISCUSSION

Discriminating topical dose assays with deltamethrin against 31 field strains of *C. lectularius* resulted in a wide variety of mortality, ranging from 15% to 100% (Figure 1) indicating resistance is widespread. A similar result was obtained against the four *C. hemipterus* strains (Figure 2), with three strains returning

≤10% mortality, while the other yielded 75% mortality. Repeat topical discriminating dose assays with imidacloprid against 30 field strains of *C. lectularius* (one strain had insufficient numbers for testing compared to deltamethrin assays) resulted in predominantly high (≥90%) mortality (Figure 3).

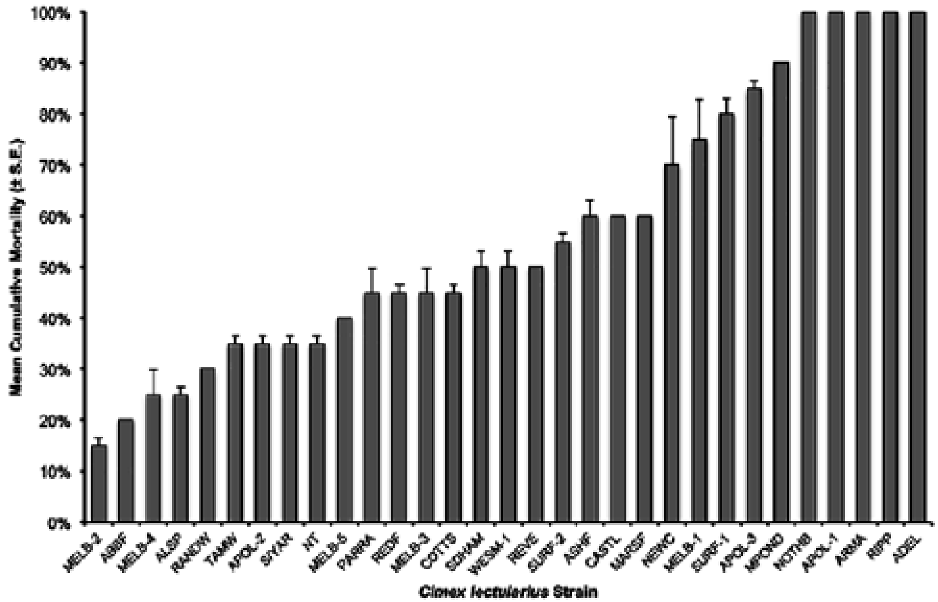

Figure 1. Mean cumulative mortality (± S.E.) of *C. lectularius* field strains treated with 2.5 g/L deltamethrin via topical application.

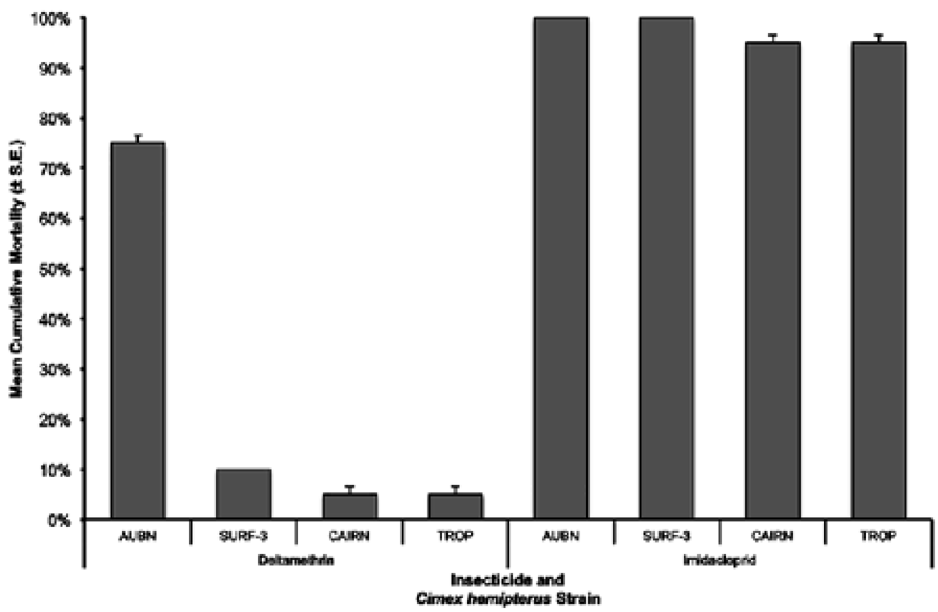

Figure 2. Mean cumulative mortality (± S.E.) of *C. hemipterus* field strains treated with either 2.5 g/L deltamethrin or 0.1 g/L imidacloprid via topical application.

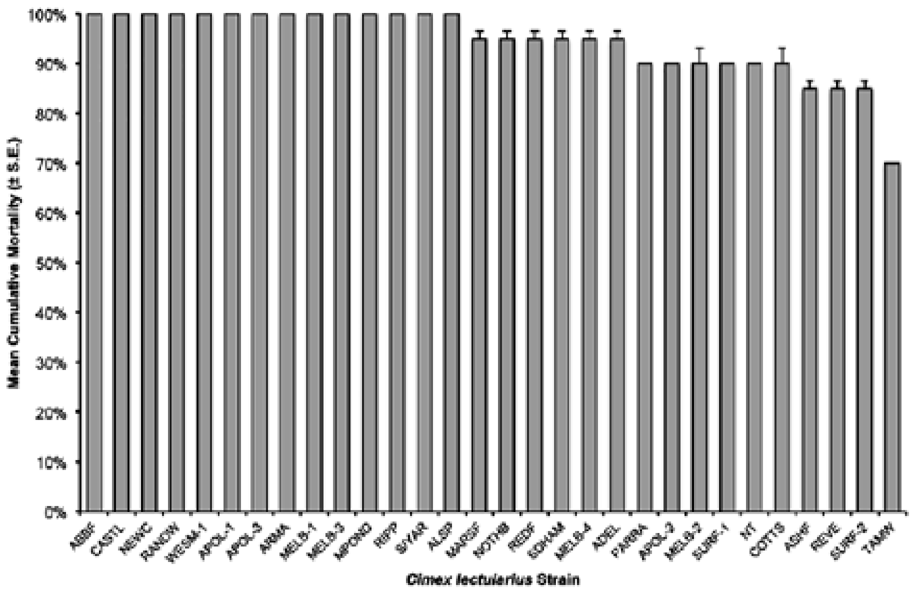

Figure 3. Mean cumulative mortality (± S.E.) of *C. lectularius* field strains treated with 0.1 g/L imidacloprid via topical application.

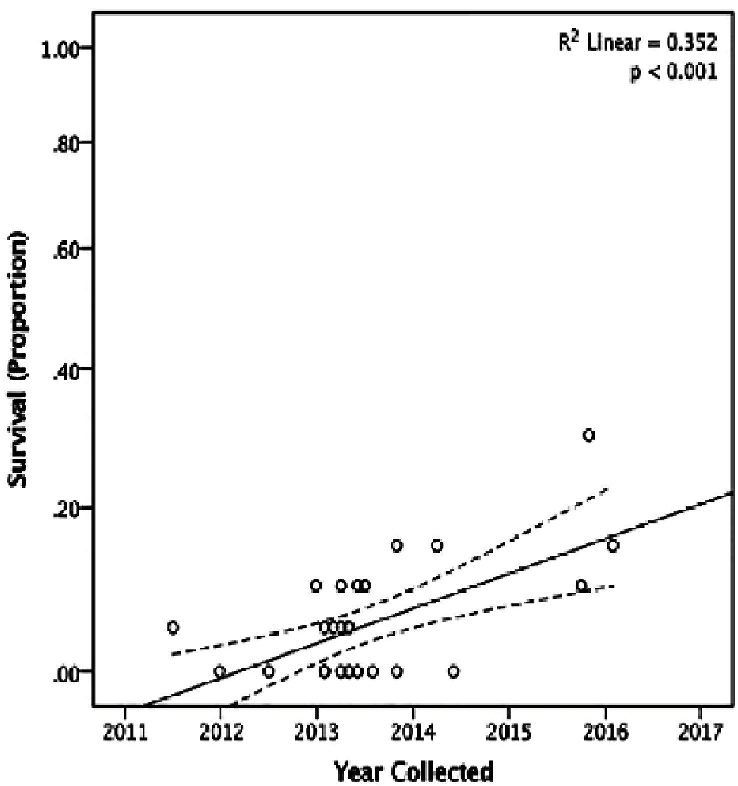

Figure 4. Linear regression (± 95% C.I.) of mean cumulative survival (± S.E.) vs. time (year collected) of *C. lectularius* field strains treated with 0.1 g/L imidacloprid via topical application.

Mortality was uniform (≥95%) with the four *C. hemipterus* strains (Figure 2) indicating imidacloprid remains a highly efficacious insecticide against current field strains of this species when applied topically. Mean control mortality across all strains was 1.25% (S.E. ± 0.45%).
Linear regression analysis of mean survival values for each strain plotted against date of collection indicated no correlation for deltamethrin with *C. lectularius* ($R^2 = 0.013$, $p = 0.536$). However, a positive correlation against collection date was returned for imidacloprid with *C. lectularius* ($R^2 = 0.352$, $p < 0.001$) (Figure 4) with the newest colonized strains being less susceptible, suggesting a degree of reduced susceptibility to the insecticide is developing in field populations. No significant trend was determined for *C. hemipterus* either for deltamethrin ($R^2 = 0.047$, $p = 0.784$) or imidacloprid ($R^2 = 0.160$, $p = 0.600$).

This study represents the most comprehensive survey to date on the topical susceptibility of modern bed bug populations in Australia to commonly used pyrethroid and neonicotinoid insecticides. The results from topical discriminating dose assays confirm that pyrethroid resistance in both *C. lectularius* and *C. hemipterus* is ubiquitous and present at a high frequency. Susceptibility to neonicotinoids predominantly remains via topical application, although there may be evidence of reduced susceptibility developing in *C. lectularius* based on a slight, but significant, trend being evident when mean mortality values were compared against the year each strain was collected. The lack of a significant regression trend for both *C. lectularius* and *C. hemipterus* against deltamethrin suggests that, despite being inherently variable from strain to strain, there is no obvious positive or negative change evident in the frequency of resistance in Australian bed bugs for the last 8 years.

Variability in the mortality achieved in both *C. lectularius* and *C. hemipterus* against deltamethrin could be due to a number of reasons and does not indicate for the absence of resistance in some strains. Notably, given *kdr*-type mutations have been found to be consistently present in field strains of both species collected post-2002 both in Australia (Dang et al., 2015b; Dang et al., 2015c) and overseas (Durand et al., 2012; Palenchar et al., 2015; Seong et al., 2010; Yoon et al., 2008; Zhu et al., 2010), variability in the mortality achieved against deltamethrin points to either the presence of some fitness-derived differences from strain to strain or, more likely, the activity of multiple resistance mechanisms. Evidence for multiple resistance mechanisms being present and widespread is supported by the fact that several *C. lectularius* strains, all with known haplotype B *kdr*-type resistance, returned cumulative mortality values between 20% and 100%, with no apparent association to other facts (such as collection date). Subsequent research on a subset of these *C. lectularius* field strains has elucidated that metabolic detoxification and cuticle thickening contribute to the observed resistance (Lilly et al., 2016a; Lilly et al., 2016b).

CONCLUSION

The development of insecticide resistance in both the *C. lectularius* and *C. hemipterus* is well established in Australia. This study confirms that field populations of *C. lectularius* are ubiquitously resistant to deltamethrin, and may be exhibiting the first signs of reduced susceptibility to neonicotinoids. *Cimex hemipterus* is even more resistant to deltamethrin, but not the neonicotinoids, although further collection and study of field specimens would be warranted beyond the four strains included in this study. Continued culturing and development of the collected field strain colonies, and subsequent elucidation of the resistance mechanisms, may provide further insights into the differences between strains. Ultimately, this will improve our understanding of how to effectively eradicate or prevent future infestations.

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