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INSECTICIDE RESISTANCE IN *CIMEX LECTULARIUS* (HEMPTERA: CIMICIDAE) IN AUSTRALIA

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Abstract Insecticide resistance in bed bugs (*Cimex lectularius* L. and *C. hemipterus* F.) is a major factor in the pest's resurgence. Various studies have demonstrated resistance to the pyrethroids, carbamates and the organophosphates. Resistance has been suspected in Australia, with anecdotal reports of poor product performance. Laboratory studies have demonstrated insecticide resistance in a previously suspected-resistant field strain of bed bugs, with substantial differences in LD_{50} values when compared to a susceptible strain. The resistance factors for each compound were: permethrin = 1.4 million, deltamethrin = 430,000, bendiocarb = 240, pirimiphos-methyl = 2.8, imidacloprid = 2.7. Resistance was confirmed in the field-collected strain with the pyrethroids and carbamates, but not the organophosphates or neonicotinoids. Professional pest management operations require the development of new strategies to combat the pest. Regulatory authorities must consider the implications of resistance to multiple insecticide groups when registering new products utilizing either existing or new modes of action.
Key words Bed bugs, pyrethroid, carbamate, organophosphate, neonicotinoids.

INTRODUCTION

Over the last two decades a major bed bug resurgence has occurred internationally. Australia has not been immune, with infestations increasing over the years 2000-2006 by 4,500% compared with pre-2000 (Doggett and Russell, 2008). A Sydney based pest management company alone reported a 700% increase in the number of treatments for the period 2000-04 (Doggett et al., 2004), with this trend continuing to an eventual 3,500% increase for the decade 2001-2011 (unpublished data).The development of resistance amongst populations of bed bugs is one of the commonly mentioned theories for their resurgence internationally (Doggett et al., 2004; Doggett et al., 2012, Myamba et al., 2002; Romero et al., 2007; Wang and Cooper, 2011).

Resistance to insecticides has become an increasingly serious international problem. Since the earliest known resistance to DDT in field populations of house flies (*Musca domestica* L.) was reported (Lindquist and Wilson, 1948; Sacca, 1947), over 540 species of insect have developed resistance to one or more insecticides (Yu, 2008a). A key problem of insecticide resistence is that it limits the available insecticidal control options that could be classed as efficacious whilst simultaneously forcing reliance on alternatives that are 'either expensive, not readily available, or not particularly effective' (Robinson and Boase, 2011). Understanding the basis and function of insecticide resistance is, therefore, critical to detecting when it evolves, in order to promptly implement effective resistance mitigation programs and develop improved control techniques to manage its effects in field situations.

Resistance in field collected *C. lectularius* to several common pyrethroid insecticides has been confirmed in the United States (Adelman et al., 2011; Koganemaru et al., 2013; Moore and Miller, 2006; Romero et al., 2007; Yoon et al., 2008). Similarly, in the United Kingdom resistance has been found to the pyrethroids and also the carbamates (Boase et al., 2006). Resistance to permethrin has also been found to be prevalent amongst infestations in Denmark, although not correspondingly so with chlorpyrifos (an organophosphate) with only low rates of resistance being detected (Kilpinen et al., 2011).

The degree of resistance detected in the above studies has been typically high, although it has since been discovered the magnitude of pyrethroid resistance may vary according to the class of the compound assayed. Type I ('non-cyano' pyrethroids such as permethrin, d-phenothrin, pyrethrins etc.) will cause predominantly lower mortality compared to Type II compounds ('α-cyano' pyrethroids such as deltamethrin, cyfuthrin, cypermethrin and fenvalerate etc.) when applied to a suspected resistant field strain (Anderson and Cowles, 2012).

Pyrethroid resistance exhibited in *C. lectularius* in the United States appears widespread and the result of multiple resistance mechanisms (Zhu et al., 2013; Zhu et al., 2010). Two *kdr*-type target site point mutations (V419L and L925I) have been identified (Yoon et al., 2008) and subsequently found to be highly prevalent, being detected (in various haplotypes) in 88% of 110 bed bug infestations sampled from across the country (Zhu et al., 2010). A *C. lectularius* strain from Richmond, VA has also been found to exhibit over expression of genes consistent with cytochrome P450 monooxygenase and carboxylesterase mediated metabolic resistance, in addition to *kdr*-type (L925I) resistance (Adelman et al., 2011). The same Richmond, VA strain has since been found to exhibit cuticular resistance with a three-fold difference in mortality when insecticide is applied via injection as opposed to topical application (Koganemaru et al., 2013). Zhu et al. (2013) also identified resistance-associated genes expressed in the epidermal layer of the integument.

In 2002, resistance in the tropical bed bug (*C. hemipterus*) to permethrin and α-cypermethrin was also recorded for the first time with a modern field infestation in Tanzanian villages where pyrethroid impregnated nets are used on beds (Myamba et al., 2002). Resistance in *C. hemipterus* has since been further confirmed with studies demonstrating resistance variously to the organochlorines, organophosphates, carbamates and pyrethroids in Sri Lanka (Karunaratne et al., 2007) and Thailand (Suwannayod et al., 2010; Tawatsin et al., 2011).

It should perhaps not be surprising that modern populations of both *C. lectularius* and *C. hemipterus* have developed resistance, particularly to the pyrethroids, given that prior to their near eradication as a public health pest in the 1960s and 1970s, bed bugs had begun to develop resistance to many insecticidal compounds (Brown, 1956; Busvine, 1958; Feroz, 1969; Gaaboub, 1971; Johnson and Hill, 1948; Sharma, 1963) and particularly those from the organochlorine family which can infer cross-resistance to the pyrethroids. There were even early reports of resistance to the pyrethrins; Busvine (1958) found two strains of bed bugs, one each of *C. lectularius* and *C. hemipterus* that had reduced susceptibility to this insecticide.

It is essential that resistance be monitored in order to detect and manage its effects for the improvement of eradication procedures and best practice in pest control. Until recently, the susceptibility status of field infestations in Australia remained unclear and could only be assumed based on anecdotal reports from pest control companies of poor product performance (Doggett and Russell, 2008) and generally low efficacy of several formulated products when

screened in laboratory trials (Lilly et al., 2009a, 2009b). Here we examine and discuss insecticide susceptibility of a field collected strain of *C. lectularius* compared to an imported known-susceptible strain in response to exposure to a wide range of insecticide groups.

MATERIALS AND METHODS

Cimex lectularius Strains

Two strains were used in this study; the Sydney strain, where the founder specimens were collected from various locations in Sydney during 2004-5 and is suspected to be resistant, and the 'Monheim' strain, a known susceptible strain that was obtained from Bayer, Germany, and originated from around the late 1960s.

Dose Response Assays

Five compounds were selected for the trial; bendiocarb, deltamethrin, permethrin, imidacloprid and pirimiphos-methyl (Table 1). These reflected the major classes of insecticides registered for bed bug control in Australia at the time of the study. The compounds were serially diluted to obtain results that produced 0-100% mortality for each insecticide and bed bug strain. Bed bugs were temporarily immobilised by securing the dorsal surface to a small strip of double-sided tape and a 1µL drop of the diluted insecticide applied to the ventral surface. The insecticide was allowed to dry, the bed bugs removed and mortality recorded at 24 hours. For each product tested, there were four replicates of ten bed bugs each and an equivalent number of controls (which received the diluent only). LD_{50} values were calculated by Probit analysis.

RESULTS AND DISCUSSION

When profiled, all compounds tested against the Monheim strain returned clear dose responses and overall high levels of insecticidal activity.

Carbamates

Bendiocarb was highly efficacious against the Monheim strain, but not against the Sydney strain. The absence of a full dose response for the Sydney strain resulted in a high level of variance within the confidence intervals but, nonetheless, the factor of difference between the calculated LD_{50} values was determined to be 239.

Pyrethroids

Permethrin and deltamethrin both performed extremely poorly when tested against the Sydney strain, with neither compound reliably achieving >60% mortality at the highest dose of 100µg/µL with the Probit estimations subsequently returning highly heterogeneous results. This was in contrast to the Monheim strain, where both compounds returned highly efficacious results. The resistance factors separating the two strains for these compounds were consequently large (1.4 million and 432,042 respectively).

Organophosphates and Neonicotinoids

Pirimiphos-methyl was efficacious against both the Sydney and Monheim strains, although the results proved significantly different. The results for imidacloprid mimicked the degree of difference (≈2.7x) between the Sydney and Monheim strains for pirimiphos-methyl. The result of our study conforms to, and extends, previous resistance studies undertaken against field and laboratory strains of *C. lectularius*.

Table 1. Insecticides selected for topical susceptibility testing against *Cimex lectularius*

Compound	Group[a]	Products Registered in Australia	Resistance Internationally	Resistance in Australia
Pirimiphos-methyl	1B	Yes	Yes (Denmark)	No
Bendiocarb	1A	Yes	Yes (UK)	Yes
Permethrin	3A	Yes	Yes (UK, US)	Yes
Deltamethrin	3A	Yes	Yes (UK, US)	Yes
Imidacloprid	4A	No[b]	No	No

[a]Group according to the Insecticide Resistance Action Committee, www.irac-online.org
[b]At the time of testing. Temprid75® (β-cyfluthrin + imidacloprid, Bayer, Melbourne, Australia) has since been released

Insecticide Resistance in Australia

Despite the above results, the full extent of resistance in bed bugs in Australia remains uncertain from this investigation. The data derived for this study was based on a strain collected between 9-10 years ago from infestations around Sydney, and hence only provides a limited 'snap-shot' of the breadth of the problem as it does not include any specimens collected from other major cities across states or territories. It is also known from quarantine interceptions that new potential populations of bed bugs have been introduced from outside Australia on a regular basis (Doggett et al., 2004), and hence obtaining a full understanding of how widespread resistance is will always be difficult. However, recent information has revealed that *kdr* mutations in *C. lectularius* are widespread across the country and all modern bed bug strains appear resistant (see Dang et al. 2014 in these proceedings). A further complicating factor is that Australia is one of the few countries to host both the common (*C. lectularius*) and tropical (*C. hemipterus*) bed bug (Doggett et al., 2003). As noted above, resistance to several insecticide classes has been previously confirmed in *C. hemipterus*, although limited susceptibility and *kdr* testing has been conducted against this species in Australia. Hence, susceptibility of the bed bug species that is likely to predominate across the northern half of the continent, and efficacy of the products available for use against them, is not fully known.

The Drivers of Insecticide Resistance in Bed Bugs

Failure of pyrethroid products to control an infestation due to underlying insecticide resistance may facilitate or increase the spread of resistant bed bugs. Bed bugs have been reported to be repelled by pyrethroids and susceptible bugs will avoid resting on areas with residual levels of deltamethrin (Kramer, 2004; Romero et al., 2009). Resistant populations may, however, still find harbourages previously populated by bed bugs attractive due to the presences of aggregation pheromones. Resistant bed bugs will also exhibit increased activity upon exposure to a sub-lethal dose and are likely to disperse or, at the very least, spend more time away from their harbourage area (Romero et al., 2009). If the levels of pyrethroid resistance confirmed within this study are encountered in a field situation, this potential response must evidently be taken into account. Repeated applications of insecticide will already be required due to poor residual efficacy of most pyrethroid products (Lilly et al., 2009a, 2009b). Consequently, treatments must extend to potential access points and areas where bed bugs

may disperse, either in avoidance of the applied insecticide or in search of a new host. In the event of continual sub-lethal exposure, resistance beyond the levels already documented are likely to develop.
There is no doubt that *kdr*-type mechanisms are contributing to the high degree of resistance. Similarly, evidence from earlier work on formulated products (Lilly et al., 2009a, 2009b) and deltamethrin (Romero et al., 2009) indicated the inclusion of piperonyl butoxide has some appreciable effect on pyrethroid efficacy, thereby suggesting the presence of cytochrome P450 monooxygenase enzymatic detoxification mechanisms (Hodgson and Levi, 1998).
In addition to these factors, supplementary mechanisms such as reduced penetration and behavioural resistance are likely to be present and acting as intensifiers of the other (potentially multiple) resistance mechanisms as above.
Reduced penetration of a toxicant through the cuticle has been known to be a resistance mechanism since it was first established in the 1960's with the pyrethrin (Fine et al., 1963), organophosphate (Forgash et al., 1962; Matsumura and Brown, 1961, 1963), carbamate (Georghiou et al., 1961), and organochlorine insecticide groups (Plapp and Hoyer, 1968; Vinson and Brazzel, 1966). It has since also been established with the pyrethroids in a wide variety of insect pests (Ahmad et al., 2006; Delorme et al., 1988; DeVries and Georghiou, 1981;Gunning et al., 1991; Noppun et al., 1989; Valles et al., 2000; Wood et al., 2010;). Ordinarily, reduced penetration does not, by itself, impart a high degree of resistance (Plapp and Hoyer, 1968; Yu, 2008), although it may nonetheless have importance by way of conferring a level of cross-resistance to a wider variety of insecticides (Oppenoorth, 1984; Plapp and Hoyer, 1968; Yu, 2008), increasing the efficiency of metabolic detoxification (Ahmad et al., 2006; Mamidala et al., 2012; Sawicki, 1970) or delaying the onset of knockdown (Scott, 1990; Wood et al., 2010) and thus has important direct implications for effectiveness of formulated products in field situations and the eradication of resistant bed bug infestations.
The status and methodology for detecting the presence or degree of reduced penetration in bed bugs is currently advancing and there is considerable scope for investigation of the structural or functional differences in resistant bed bug strains. Evidence from very recent molecular analysis of several *C. lectularius* strains point toward changes in protein-expression and manifestation of other resistance mechanisms in the cuticle being strongly indicative of reduced penetration and/or cuticle thickening (Adelman et al., 2011; Koganemaru et al., 2013; Mamidala et al., 2012; Zhu et al., 2013).
Utilising the framework set by the above molecular research on *C. lectularius* it is axiomatic that research specifically examining cuticle thickness and the dynamics of insecticide penetration should be undertaken on resistant bed bug strains. Changes in cuticle thickness, the lipid composition and passage of insecticides, if combined with expression of detoxifying enzymes or target-site insensitivity, may have important consequences for the use of formulated insecticides in control of infestations.
A final mechanism, behavioural resistance, similarly needs to be considered as it may enable insects to avoid a lethal dose through stimulus-dependent hypersensitivity or hyperirritability. As a resistance mechanism, behavioural resistance is less well understood than the physiological resistance, although it has been demonstrated that behaviourally resistant insects respond to lower concentrations of insecticide than resistant strains (Silverman and Bieman, 1993; Young and McMillian, 1979), indicating a potential physiological change in receptor sensitivity. Common bed bugs have been found to be repelled and irritated by pyrethroid residues, but will otherwise overcome this repellency in the presence of a host, harbourage, and/or aggregation pheromone. Thus, there is the potential that if bed bugs have attained the tendency to be hypersensitive to such residues, that such a behavioural response may also intensify other pre-existing physiological resistance mechanisms.

CONCLUSION

The evolution of insecticide resistance is the expected result of their repeated use. A high degree of resistance in *C. lectularius* has now been confirmed in Australia, and an early warning provided that resistance has developed in the previously effective carbamate insecticide group. The inability to control bed bugs with existing products and insecticides will necessitate a rethinking of control methodologies employed against this resurgent pest. Integrated pest management in the form of a solid understanding of the ecology, biology, risks and effective treatment options associated with a bed bug infestation will be required for successful eradication. Products that employ new modes of action, or the re-labelling and reformulation of existing products will be needed, along with greater development and utilisation of efficacious non-chemical methods such as mattress encasements, steam, vacuuming and monitoring devices, and the adherence of pest managers to industry standards or codes of practice on bed bug management. Further susceptibility research and exploration of resistance mechanisms, combined with the inclusion of *C. hemipterus* in such testing, is fundamental to both a better understanding of the causes of the resurgence and our ability to effectively counter infestations. Without an integrated approach control of bed bugs will continue to be difficult.

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