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Special Collection: Perspective on Biology and Management of Bed Bugs

The Efficacy of a Pyrethroid-impregnated Mattress Liner on Multiple International Strains of *Cimex lectularius* (Hemiptera: Cimicidae) and *Cimex hemipterus* (Hemiptera: Cimicidae)

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Abstract

Modern bed bugs are resistant to multiple insecticide classes, particularly the pyrethroids. The efficacy of pyrethroid-impregnated mattress liners marketed for bed bug management has been variable. This study evaluated the efficacy of a permethrin-impregnated mattress liner, ActiveGuard, against 24 bed bug strains, consisting of both *Cimex hemipterus* (F.) and *Cimex lectularius* L. A 'mat assay', employing an allethrin-impregnated mat, was used to establish the pyrethroid resistance profile of all strains. Three experiments were conducted to evaluate the effect of ActiveGuard exposure on bed bug knockdown: 1) exposing the bed bugs continuously on the liner for up to 24 d, 2) holding the bed bugs on the liner for either 4 or 6 h, and 3) placing a noninsecticide treated fabric above the liner with the bed bugs held continuously on top. Our results indicated that all modern strains (collected within the last 15 years during the current resurgence) were pyrethroid-resistant, although the magnitude of resistance was highly variable between strains. In the continuous exposure study, an incomplete knockdown was recorded for most modern bed bug strains, with some having no knockdown even up to 7 d of constant exposure. In the 4 or 6 h exposure study, the level of knockdown was reduced even further, and very few bed bugs were knocked down in the double fabric study. The results of this study indicate that pyrethroid-impregnated mattress liners are not likely to be effective in the management of most modern bed bug infestations involving either *C. hemipterus* or *C. lectularius*.

Key words: ActiveGuard, efficacy, *Cimex hemipterus*, *Cimex lectularius*, resistance

The global resurgence of both the tropical bed bug, *Cimex hemipterus* (F.) (Hemiptera: Cimicidae), and common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), has demanded more efficacious and cost-effective management solutions (Doggett et al. 2012, 2018a). Many products have come onto the market during this resurgence, however all have limitations, as evident by the continuing proliferation of bed bug infestations (Doggett and Feldlaufer 2018). Modern bed bugs have developed an extremely high levels of insecticide resistance (Dang et al. 2017, Romero 2018). In studies where either bed bug species have been assayed for resistance, all strains have been found to be highly resistant to the pyrethroids (Myamba et al. 2002, Boase et al. 2006, Romero et al. 2007, Tomita 2010, Kilpinen et al. 2011, Dang et al. 2014, Palenchar et al. 2015, Balvin and Booth 2018, Lilly et al. 2018, Holleman et al.

2019, Punchihewa et al. 2019, Cho et al. 2020, Vander Pan et al. 2020, Zhao et al. 2020, Akhoundi et al. 2021, Deku et al. 2021, Dang et al. 2021, Soh and Veera Singham 2021). Furthermore, resistance in modern bed bugs has been reported to other insecticide classes including the neonicotinoids, organochlorides, carbamates, organophosphates, aryl pyrroles, and a reduced susceptibility to diatomaceous earth dust (Dang et al. 2017, Romero 2018).

One of the most debatable bed bug management strategies is the use of pyrethroid-impregnated fabrics, which are marketed as mattress covers, liners, and even as the material used to manufacture the mattress itself (Doggett and Feldlaufer 2018). A popular pyrethroid-impregnated liner used for bed bug management in the United States is the ActiveGuard mattress liner (Allergy Technologies, Ambler, PA) containing 1.64% permethrin. This product was initially developed to control house dust mites but has lately been marketed for bed bug control (Ballard 2008). In assessing the efficacy of this product against bed bugs, Jones et al. (2013) tested four strains of *C. lectularius* that were continuously exposed to the ActiveGuard mattress liner and reported that three strains died within 24 h. However, the surviving strain (Marcia) experienced only 22% mortality after 10 d of continuous exposure. In another continuous exposure evaluation using the pyrethroid-resistant 'Sydney' strain of *C. lectularius*, less than 20% mortality was recorded after 24 h. Subsequently, the mortality did not increase even after 16 d of continuous exposure (Doggett et al. 2011). More recent research has evaluated the efficacy of Aprehend (ConidioTec LLC, State College, PA), a fungal-based biopesticide containing *Beauveria bassiana*, against *C. lectularius* when applied to an ActiveGuard mattress liner (Shikano et al. 2019). The researchers found that ActiveGuard did not contribute to the bed bug mortality. In spite of these reported studies, the number of modern bed bug strains evaluated for susceptibility to permethrin-impregnated mattress liners has been limited, and none of the studies have assessed the mattress liner for the control of *C. hemipterus*.

The use of pyrethroid-impregnated mattress products may have added consequences for the management of bed bug infestations. Bed bug management is a costly process, and many organizations impacted by bed bugs have limited fiscal resources (Doggett et al. 2018b). Therefore, they must optimize these restricted financial resources to ensure that the best possible management options are employed.

In this multi-institutional study, we evaluated the ActiveGuard mattress liner for the control of 24 strains of bed bugs. These strains included both *C. lectularius* and *C. hemipterus* species originating from Australia, Malaysia, Sweden, the United Kingdom, and the United States, with 21 collected during the modern resurgence. All strains were assayed for their pyrethroid resistance via the 'mat' assay (Dang et al. 2015a) prior to testing on the mattress liner. As bed bugs are known to move about whilst searching for either a host or harborage (Romero et al. 2010), three experimental designs were conceived in order to assess the efficacy of the mattress liner to simulate real-world conditions. Firstly, experiments were undertaken to evaluate the efficacy of bed bugs held continuously on the mattress liner. Secondly, as the bed bugs move and may contact the liner only intermittently, a trial was conducted that examined the effectiveness of short-term exposure, where the insects were held on the liner for either 4 or 6 h. Finally, because it is not recommended that permethrin-impregnated liners be in direct contact with the human skin, a third test examined the efficacy of the liners when covered by a noninsecticide impregnated fabric. The purpose of the overall study investigations was to determine if pyrethroid-impregnated mattress liners should, or should not be, recommended as part of an overall bed bug management program.

Materials and Methods

Bed Bugs

Twenty-four bed bug strains were used in the permethrin-impregnated mattress liner evaluation trials, including seven strains of *C. hemipterus* and 15 strains of *C. lectularius*, with two insecticide susceptible *C. lectularius* strains as controls. The bed bugs strain collection dates and locations are listed in Table 1. The insecticide susceptible strains used as the controls included the Monheim strain (in the Australian, British, and Malaysian studies) and the Harlan ('Fort Dix') strain (US studies). As no insecticide susceptible strain of *C. hemipterus* could be sourced worldwide, the Monheim strain of *C. lectularius* was used as the control. This strain has been used in other insecticide investigations involving *C. hemipterus* (Dang et al. 2015b, Leong et al. 2020a, b, Dang et al. 2021). None of the bed bug strains used in this study had undergone any laboratory induced selection for insecticide resistance.

The Australian bed bug strains were reared under laboratory conditions of ~25°C, ~70% RH, and a photoperiod of 12:12 (L:D) h. The bed bugs were blood-fed once per week on specific-pathogen-free anesthetized rats. The Australian field strains used in this study possess a range of resistance profiles that had been determined by previous research, which categorized resistance levels when strains were exposed to a discriminating dose of deltamethrin (Lilly et al. 2018). The Adelaide and Ripponlea strains had low-level resistance, the Parramatta, Tamworth, Sydenham, and Newcastle strains demonstrated mid-level resistance, and the Alice Springs and Melbourne strains showed high resistance levels (Lilly et al. 2018). The Newcastle strain has extremely low fecundity and thus fewer replicates of bed bugs were used in assaying this strain. As this is a mid-level resistant strain, we felt it important to include the data, even if limited. Mixed-sex adults of each strain were used in this study, and bed bugs were offered the opportunity to feed to repletion 5–7 d prior to testing.

The Malaysian bed bugs strains were reared under laboratory conditions of ~27°C, ~70% RH, and a photoperiod of 12:12 (L:D) h. The Australian originating Queensland strain was reared and tested in Malaysia. The bed bugs were fed with freshly drawn rabbit blood in lithium heparin tubes (Vacutest Kima srl, Arzergrande [PD], Italy) once per week using the Hemotek membrane feeding system (Discovery Workshops, Accrington, UK). With the exception of the Kuala Lumpur (collected in 2005) and Madam Mo (collected in 2016) strains, all other Malaysian strains were collected from infested houses in 2015. Mixed-sex adults of each strain were used in this study, and bed bugs were offered to feed to repletion 7–9 d prior to testing.

The UK bed bugs strains were reared under laboratory conditions of ~27°C, ~70% RH, and a photoperiod of 12:12 (L:D) h. The bed bugs were allowed to feed once per week on a human volunteer (author RN). The populations selected for this study had a range of resistance profiles correlating with their time in culture. The L1 strain, collected in c.1970, is similar to the Monheim strain in terms of its susceptibility to pyrethroids and other modern insecticides (authors RN&AN, unpublished data). Previous studies have found moderate levels of pyrethroid resistance in the F4 (UK) and Malmö (Sweden) strains, as well as high levels of pyrethroid resistance in the London N7 (UK) strain. Mixed-sex adults of each strain were used in this study, and bed bugs were offered to feed to repletion 5–7 d prior to testing.

The US bed bug strains were reared under laboratory conditions of ~25°C, ~70% RH, and a photoperiod of 12:12 (L:D) h. The bed bugs were fed once per week on defibrinated rabbit blood (Hemostat

Table 1. Bed bug strains used in the study, their location of origin, date of collection, testing date, and resistance level

Strain	Species	Collection location	Collection date	Testing date date	Resistance level[a]
Adelaide	*C. lectularius*	Adelaide, Australia	2013	2018	M
Ripponlea	*C. lectularius*	Ripponlea, Australia	2013	2018	N
Parramatta	*C. lectularius*	Parramatta, Australia	2012	2018	VH
Tamworth	*C. lectularius*	Tamworth, Australia	2015	2018	VH
Sydenham	*C. lectularius*	Sydenham, Australia	2013	2018	VH
Newcastle	*C. lectularius*	Newcastle, Australia	2013	2018	VH
Alice Springs	*C. lectularius*	Alice Springs, Australia	2013	2018	VH
Melbourne	*C. lectularius*	Melbourne, Australia	2013	2018	VH
Queensland	*C. hemipterus*	Queensland, Australia	2004	2018	VH
Kuala Lumpur	*C. hemipterus*	Kuala Lumpur, Malaysia	2005	2018	H
Bukit Mertajam	*C. hemipterus*	Bukit Mertajam, Malaysia	2015	2018	VH
Saujana	*C. hemipterus*	Penang, Malaysia	2015	2018	VH
Krystal Point	*C. hemipterus*	Krystal Point, Malaysia	2015	2018	VH
Madam Mo	*C. hemipterus*	Penang, Malaysia	2016	2018	VH
Tanjong Tokong	*C. hemipterus*	Tanjong Tokong, Malaysia	2015	2018	VH
F4	*C. lectularius*	London, UK	2012	2018	N
L1	*C. lectularius*	London, UK	c.1970	2018	N
London N7	*C. lectularius*	London, UK	2019	2018	VH
Malmö	*C. lectularius*	Malmö, Sweden	2018	2018	M
Epic Center	*C. lectularius*	Cincinnati, OH, USA	2009	2019	VH
Richmond	*C. lectularius*	Richmond, VA, USA	2008	2019	M
Vinton	*C. lectularius*	Vinton, VA, USA	2019	2019	VH
Harlan	*C. lectularius*	Fort Dix, NJ, USA	1973	2019	N
Monheim	*C. lectularius*	Germany	c.1970	2019	N

[a]Resistance level determined by the results of the mat assay herein and using the classification of Leong et al. (2020b). N = No resistance, L = Low, M = Moderate, H = High, VH = Very High.

Laboratories, Dixon, CA) using an artificial feeding system, maintained at 35.5°C by circulating hot water. Mixed-sex adults of each strain were used in this study, and bed bugs were offered the opportunity to feed to repletion 5–7 d prior to testing.

For the experiments, five replicates of ten bed bugs were used. However, due to the limited number of individual bed bugs in some resistant strains, fewer bugs were tested. The numbers of bed bugs used in each trial are detailed in Tables 2-6.

Mattress Liner

Four ActiveGuard (1.64% permethrin) queen-sized mattress liners were purchased online from 'Do-It-Yourself Pest Control' (cat. no.: SKU: 5688200103Queen). One liner was sent to each respective testing laboratory. The liners were used within the two-year use-by-date recommendation. No nonpermethrin impregnated liners were available for use as a control fabric. Instead, a fabric of similar composition (100% polyester, unbranded) was purchased (by SLD) and swatches were sent to the participating laboratories for use as controls, and for covers in the double-fabric study.

Experimental Procedures

Four experiments were conducted: 1) Determination of resistance levels using the 'mat assay' method (Dang et al. 2015a), 2) A continual exposure study whereby bed bugs were constantly held on the mattress liner, 3) A study whereby bed bugs were held on the mattress liner for either 4 or 6 h and transferred to a noninsecticide impregnated fabric, and 4) A study whereby a second noninsecticide treated fabric was positioned over the mattress liner (double-fabric study), and bed bugs placed onto the upper fabrics and held in place continuously. For these experiments, knockdown was recorded for different periods per each laboratory, as detailed below. A bed bug was considered knocked-down when no coordinated movement was observed, or the insect could not correct its position.

Insecticide Resistance Testing

Each bed bug strain was tested to determine its pyrethroid resistance profile using the method described by Dang et al. (2015a). This procedure was employed due to the ease and speed of the methodology, and that few bed bugs need to be used for a broad indication of resistance levels. Briefly, the 'mat assay' employs a mosquito mat impregnated with 40 mg d-allethrin/pad (Mortein Odourless Mozzie Zapper). In the study, ten bed bugs were placed into a clean plastic vial (5 ml). The vial's opening was covered with a freshly opened mat (each mat is sold sealed in a foil pack). The vial was inverted onto the mat to ensure that the bed bugs remained in constant contact with the mat surface. The vial was then upturned at regular time intervals, to observe the knockdown of the bed bugs as detailed below. Bed bugs that dislodged from the mat when the vial was inverted, and showed no coordinated movement upon gentle shaking, were considered knocked-down. Each mat was used for one replication and discarded. Control vials were covered with filter paper and were treated as described for the mosquito mat assays. Each mat assay had five replicates.

For the Australian trials, knockdown was recorded every hour for up to six hours, and then at every 24 h for up to 7 d. For the Malaysian trials, the knockdown response of bed bugs was observed at intervals of 5 min during the first 40 min for Monheim susceptible strain (MY), at intervals of 30 min from 4 – 10 h postexposure forall *C. hemipterus* field strains at intervals of 2 h from 4 to 24 h, and there on at intervals of 6 h for up to 4 d. For the UK trials, the knockdown response of bed bugs was observed hourly for up to 4 h and then daily up to 21 d. For the US trials, knockdown was monitored for every 10 min for up to 1 hr, then hourly every 6 h for 24 h, and then daily for up to 4 d.

Continuous Exposure Experiment

Swatches of the mattress liner were cut out and fixed onto the base of a 9 cm diameter Petri dish with double-sided tape. Ten bed bugs

of mixed-sex were placed into the Petri dish, contacting the mattress liner, thus achieving constant exposure. Control replicates were placed on untreated fabric. Each bed bug strain had five replicates, except for some of the more highly resistant strains, where numbers in the colony were limited. Bed bugs that did not show coordinated movement when gently probed were considered knocked-down, while those that showed no response were considered dead. As it is not possible to determine time of death/knockdown, both were combined in the results for this and the remainder of the experiments as mortality. For the Australian trials, mortality recordings were undertaken hourly for up to 6 h and then at 24 h post-exposure daily for 7 d. In the Malaysian trials, bed bug mortality was recorded daily for 4 d. For the UK trials, mortality was monitored daily for 21 d. For the US trials, mortality was monitored daily for 24 d.

Four or Six hour Exposure Experiment

Bed bugs in groups of 10 were placed directly onto the mattress liner for either 4 or 6 h as per the previous experiment. After the given time period, the insects were removed and placed onto a noninsecticide impregnated control fabric and mortality recorded. Control replicates were placed on untreated fabric. Each treatment for each strain was repeated five times. For the Australian trials, 4 h of exposure to the mattress liner was used. The Australian study was only undertaken on the Monheim, Adelaide, and Ripponlea strains, as the mattress liner in the continuous exposure experiment failed to cause any knockdown or mortality in the other strains. With the strains tested, mortality recordings were undertaken hourly for up to 6 h, and then at 24 h post-exposure daily for 7 d. For the Malaysian trials, 4 h of exposure to the mattress liner was used, and mortality was observed daily for 4 d. For the US trial, the bed bugs were kept on the mattress liner for 6 h before being transferred to the control fabric. The bed bugs were monitored for mortality daily, for 24 d. The UK group did not undertake the 4 or 6 h exposure study due to a lack of bed bugs.

Double Fabric Experiment

A layer of untreated (control) fabric was placed on top of the mattress liner, and 10 bed bugs per strain (with five replicates) were placed onto the control fabric and held continuously. The study was only undertaken on the Monheim and Ripponlea strains for the Australian trials, as the mattress liner in the 4 h exposure experiment failed to produce any knockdown or mortality in the other strains. Mortality recordings were undertaken hourly for 6 h, and then at 24 h postexposure daily for 7 d. For the Malaysian trials, the mortality responses of bed bugs were observed daily for 4 d. For the UK trials, mortality was monitored daily for 21 d. The US group did not undertake the double fabric study due to a lack of bed bugs.

Data Analysis

For the insecticide resistance assay, control knockdown was corrected using Abbott's (1925) formula. Data from all replicates were pooled and subjected to probit analysis using Polo Plus (Robertson et al. 2017). Knockdown at selected time intervals was chosen and compared. Resistance ratios (RR_{50}) were obtained by dividing the KT_{50} of the specified strain with that of the KT_{50} of the susceptible laboratory strain (i.e., the Monheim strain for the Australian, Malaysian, and UK studies, and Harlan strain for the US study). KT_{50}s were considered significantly different ($P < 0.05$) from one another based on nonoverlapped of 95% fiducial limits. The classification of resistance followed that of Leong et al. (2020b): ≤1 = no resistance; >1 to ≤5 = low resistance; >5 to ≤10 = moderate resistance; >10 to ≤50 = high resistance; >50 = very high resistance. For the experiments using the mattress liner, the mean survival times were determined by analyzing knockdown data using Kaplan–Meier survival analysis, and the survivorship curves were compared with the corresponding susceptible strains for each laboratory using Mantel–Cox log-rank test in SPSS version 27.0 (IBM Corporation, Armonk, NY).

Results

Insecticide Resistance Testing

Table 2 details the susceptibility of all bed bug strains in the mat assay. Control mortality throughout the entire experiments for all strains ranged from 0 to 6%. In the Australian trials, all susceptible Monheim strain bed bugs were knocked down within 3 h in the mat assay, while all modern field *C. lectularius* strains showed pyrethroid resistance when subjected to the mat assay, although the degree of resistance was variable (RR_{50} ranged from 2.3 to 1123.4). With the Newcastle, Alice Springs, and Melbourne strains, no mortality was recorded despite the 7 d continuous contact with the d-allethrin impregnated mat, and less than two knockdowns were recorded with the Sydenham and Tamworth strains. Only the Adelaide strain had 100% knockdown, and this took 3 d of constant exposure. The Ripponlea strain was the least resistant strain, with ~2x RR_{50} compared to the Monheim strain. Based on the classification of Leong et al. (2020b) as determined by the 'mat' assay, the Ripponlea strain had 'low' resistance, the Adelaide strain had 'moderate', and the remaining strains had 'very high' resistance.

In the Malaysian trials, all modern field *C. hemipterus* strains showed pyrethroid resistance when subjected to the mat assay (RR_{50} ranged from 35.4 to >300) (Table 2). The 96 h knockdown of all tested *C. hemipterus* strains ranged from 8 to 96%. KT_{50} values for the Queensland and Saujana strains failed to be generated because less than 50% knockdown had occurred within 96 h, thus demonstrating 'very high' resistance levels. Kuala Lumpur, the least resistant strain (RR_{50} = 35.4), demonstrated a 'high' level of resistance towards the d-allethrin mat. Bukit Mertajam, Krystal Point, Madam Mo, and Tanjong Tokong strains demonstrated 'very high' resistance levels. At 72 h posttreatment, mortality in the Queensland, Bukit Mertajam, Saujana, Madam Mo, and Tanjong Tokong strains ranged from 8 – 96%.

When evaluating the UK modern field bed bug strains, the resistance ratio ranged from 2.0 to >1,575. In the L1 strain, 100% knockdown was achieved in 24 h (Table 2). For the F4 and Malmö strains, knockdown at 48 h was 92% and 76%, respectively. For the London N7 strain, knockdown was only 20% despite the bed bugs being in continuous contact with the mat for 21 d. The L1 showed 'no' resistance, the F4 strain had 'low' resistance, the Malmö had 'moderate' resistance, and the N7 had 'very high' resistance.

In the US trials, the knockdown of the susceptible Harlan strain reached 94% after 1 d in the mat assay and 74% in the Richmond strain (Table 2). The Vinton and Epic Center strains were held on the mats continuously for 4 d, and knockdown reached 32 and 40%, respectively. RR_{50} was 4.5 for Richmond strain, and >185 and 256.8 for the Vinton and Epic Center strains, respectively. The Richmond strain had 'moderate' resistance, while the Vinton and Epic Center both had 'very high' resistance.

Continuous Exposure Experiment

Table 3 details the mean survival times and mortality of both species in the continuous mattress liner exposure experiment. The mean survival time of all field strains was significantly longer ($P < 0.05$) than their corresponding susceptible strain.

Table 2. The susceptibility of *C. hemipterus* and *C. lectularius* bed bug strains used in this study to d-allethrin impregnated mats

Species strain (country)	*n*	KT_{50} (95% fiducial limit) (in hour)	KT_{95} (95% fiducial limit) (in hour)	Slope ± SE	χ^2 (df)	RR_{50}	Cumulative % mortality		
							24-h	48-h	72-h
C. lectularius									
Monheim susceptible (MYS)	50	0.3 (0.3–0.3)	0.5 (0.5–0.5)	7.2 ± 0.6	4.1 (13)	1.0	100	100	100
Monheim susceptible (AUS)	50	[a]				–	100	100	100
Monheim susceptible (UK)	50	[a]				–	100	100	100
Harlan susceptible (US)	50	0.5 (0.3–0.8)	20.1 (10.3–63.0)	1.0 ± 0.2	4.0 (5)	1.0	100	100	100
Adelaide (AUS)	50	9.7 (8.7–11.0)	40.3 (32.3–53.1)	2.7 ± 0.2	1.3 (5)	30.3	84	98	100
Ripponlea (AUS)	50	0.73 (0.1–1.4)	9.3 (4.8–153.0)	1.5 ± 0.2	15.0 (4)	2.3	96	96	96
Newcastle (AUS)	10	>72	>72			>525	0	0	0
Sydenham (AUS)	50	>168	>168			>525	0	0	0
Parramatta (AUS)	50	359.5 (229.3–963.2)	n/a	1.0 ± 0.2	1.8 (5)	>1123.4	14	20	22
Tamworth (AUS)	50	>168	>168			>525	0	0	10
Alice Springs (AUS)	50	>168	>168			>525	0	0	0
Melbourne (AUS)	50	>168				>525	0	0	0
F4 (UK)	50	[b]				–	88	–	–
LI (UK)	50	0.6 (0.4–0.9)	2.97 (2.2–5.1)	2.5 ± 0.6	0.6 (3)	2.0	100	100	–
London N7 (UK)	50	>504	>504			>1575	10	10	–
Malmö (Sweden)	50	6.8 (4.1–11.3)	241.13 (88.8–1713.5)	1.1 ± 0.1	7.0 (5)	21.3	38	38	–
Vinton(US)	50	>96	>96			>185	8	–	–
Epic Center (US)	50	133.5 (52.3–963.0)	>133	0.6 ± 0.1	5.3 (6)	256.8	36	–	–
Richmond (US)	50	3.9 (2.7–6.2)	71.1 (29.3–393.4)	1.3 ± 0.1	9.2 (6)	4.5	76	–	–
C. hemipterus									
Kuala Lumpur (MYS)	50	11.4 (10.4–12.3)	24.0 (20.1–29.8)	5.3 ± 0.7	1.4 (3)	35.4	–	–	96
Bukit Mertajam (MYS)	50	45.0 (36.3–57.1)	641.0 (359.8–1691.7)	1.4 ± 0.2	5.6 (6)	140.5	38	48	50
Saujana (MYS)	50	>96	>96			>300	12	28	34
Krystal Point (MYS)	50	23.9 (16.1–32.8)	154.9 (87.7–579.0)	2.0 ± 0.2	10.5 (5)	74.7	64	70	78
Madam Mo (MYS)	50	37.8 (32.3–45.3)	>96	1.5 ± 0.2	4.4 (11)	118.2	40	56	62
Tanjung Tokong (MYS)	50	36.0 (29.6–47.9)	>96	1.8 ± 0.2	3.7 (8)	112.6	40	50	50

KT = Knockdown time.
[a]Insufficient data collected as all test insects died within an hour.
[b]Insufficient data collected as a large portion of the test insects died within an hour.

When evaluating the Australian strains, not all the bed bugs were knocked-down or killed, even with 7 d of continuous exposure to the mattress liner. For most Australian strains, mortality was less than 10%. No bed bugs in Sydenham or Melbourne strains were knocked-down or killed, hence the mean survival time could not be generated. Control mortalities ranged from 2 to 14% at 7 d posttreatment.

The Malaysian trials also showed poor performance of the mattress liner to kill the *C. hemipterus* strains (Table 3). With exception of the Madam Mo strain, all strains showed mean survival times exceeding 60 h. The Queensland, Kuala Lumpur, Bukit Mertajam, Saujana, Krystal Point, and Tanjong Tokong strains recorded mortality ranging from 2 to 52% after 4 d of continuous exposure (Table 3). No control mortality was recorded for the Malaysian trials.

In the UK trial, all susceptible Monheim and L1 strain bed bugs showed mean survival times below 3.5 h with the continuous exposure study (Table 3). At 21 d of continuous exposure, the F4, Malmö, and London N7 strains experienced knockdown and mortality levels of 2%, 46%, and 0%, respectively. Mean survival times for Malmö and F4 were >300 h. No control mortality was observed for the UK trials.

In the US study, 100% mortality was achieved with the bed bugs of the Harlan strain within 24 h. After 24 d of constant contact with the mattress liner, knockdown and mortality for the Richmond, Vinton, and Epic Center strains was recorded as 84%, 32%, and 52% respectively. There was minimal control mortality up to 7 d, however by 24 d posttreatment for the Harlan strain, control mortality was 22% and ranged from 14 to 36% for the field strains.

Four or Six hour Exposure Experiment

All susceptible Monheim bed bugs were knocked-down within 3 h of exposure to the mattress liner and hence not transferred to the noninsecticide-treated surface. The Australian Ripponlea strain only experienced 46% mortality after 7 d. The Parramatta strain, which was highly resistant, only had 4% mortality after 7 d (Table 4). Mean survival times for all field strains evaluated were significantly longer ($P < 0.05$) than that of the Monheim susceptible (AUS) strain. None of the Adelaide strain was killed. Since the mattress liner failed to cause any significant mortality in the other Australian modern field strains used in the previous continuous exposure study, they were not included in this 4 h exposure evaluation. Control mortality ranged from 0 to 10% at 7 d posttreatment.

In the Malaysian trials, low mortalities (0–18%) were observed when the adults of modern field *C. hemipterus* strains (except the Madam Mo strain) were exposed to the mattress liner for

Table 3. Mean survival times and mortality of *C. lectularius* and *C. hemipterus* in continuous ActiveGuard mattress liner exposure experiment

Species Strain (country)	*n*	Mean survival time (h)[a]	Std. error	95% CI	Cumulative % mortality 4-d	7-d	21-d	24-d
C. lectularius								
Monheim susceptible (MY)	50	0.4	0.0	0.3–0.4	100	–[c]	–[c]	–[c]
Monheim susceptible (AUS)	50	1.1	0.1	1.0–1.3	100	–[c]	–[c]	–[c]
Monheim susceptible (UK)	50	2.0	0	2.0–2.0	100	–[c]	–[c]	–[c]
Harlan susceptible (US)	50	24.0	0	24.0–24.0	100	–[c]	–[c]	–[c]
Adelaide (AUS)	50	–[b]			10	16	–[c]	–[c]
Ripponlea (AUS)	50	46.6*	9.0	29.0–64.1	78	82	–[c]	–[c]
Newcastle (AUS)	40	–[b]			8	10	–[c]	–[c]
Parramatta (AUS)	50	–[b]			4	4	–[c]	–[c]
Sydenham (AUS)	50	–[b]			0	0	–[c]	–[c]
Tamworth (AUS)	50	–[b]			5	5	–[c]	–[c]
Alice Springs (AUS)	50	–[b]			4	4	–[c]	–[c]
Melbourne (AUS)	50	–[b]			0	0	–[c]	–[c]
F4 (UK)	50	504.0*	0	504.0–504.0	–[c]	0	2	–[c]
L1 (UK)	50	3.5*	0.2	3.0–3.9	–[c]	100	–[c]	–[c]
London N7 (UK)	50	–[b]			–[c]	0	0	–[c]
Malmö (Sweden)	50	316.3*	32.2	253.1–379.4	–[c]	40	46	–[c]
Vinton (US)	50	503.5*	16.0	472.2–534.9	0	2	–[d]	–[d]
Epic Center (US)	50	496.3*	23.7	449.9–542.8	10	10	22	32
Richmond (US)	50	174.7*	30.9	114.1–235.3	64	64	–[d]	–[d]
C. hemipterus								
Queensland (AUS)	50	94.0*	1.7	90.8–97.4	10	–[c]	–[c]	–[c]
Kuala Lumpur (MY)	50	89.3*	2.6	84.2–94.5	20	–[c]	–[c]	–[c]
Bukit Mertajam (MY)	50	82.2*	3.5	75.4–89.0	38	–[c]	–[c]	–[c]
Saujana (MY)	50	83.0*	2.7	77.6–88.6	34	–[c]	–[c]	–[c]
Krystal Point (MY)	50	–[b]			2	–[c]	–[c]	–[c]
Madam Mo (MY)	50	3.9*	0.4	3.1–4.8	100	–[c]	–[c]	–[c]
Tanjung Tokong (MY)	50	66.3*	4.8	56.8–75.8	52	–[c]	–[c]	–[c]

An * indicates that the strain's mean survival time is significantly different from that of the corresponding susceptible strain (Mantel–Cox log-rank test, $P < 0.05$).
[b]Analysis not possible due to no or low mortality even at 7 or 21 d post-treatment.
[c]Not available as mortality was already 100% or not scored.
[d]Control mortality was >20% and thus mortality was not calculated.

Table 4. Mean survival times and mortality of *C. lectularius* and *C. hemipterus* in ActiveGuard mattress liner 4-hour exposure experiment

Species Strain (country)	*n*	Mean survival time (h)[a]	Std. error	95% CI	Cumulative % mortality 1-d	4-d	7-d
C. lectularius							
Monheim susceptible (MY)	50	0.4	0.0	0.3–0.4	100	–[c]	–[c]
Monheim susceptible (AUS)	50	5.0	0	5.0–5.0	100	–[c]	–[c]
Adelaide (AUS)	50	–[b]			0	0	0
Ripponlea (AUS)	50	72.3*	9.7	53.2–91.4	20	34	46
Parramatta (AUS)	50	–[b]			2	4	4
C. hemipterus							
Queensland (AUS)	50	–[b]			0	0	–[c]
Kuala Lumpur (MY)	50	94.1*	1.7	90.7–97.5	2	18	–[c]
Bukit Mertajam (MY)	50	91.9*	2.2	87.7–96.2	2	16	–[c]
Saujana (MY)	50	–[b]			0	6	–[c]
Krystal Point (MY)	50	–[b]			0	0	–[c]
Madam Mo (MY)	50	4.1*	0.3	3.5–4.7	80	100	–[c]
Tanjung Tokong (MY)	50	–[b]			10	10	–[c]

An * indicates that the strain's mean survival time is significantly different from that of the corresponding susceptible strain (Mantel–Cox log-rank test, $P < 0.05$).
[b]Analysis not possible due to no mortality even up at 4 or 7 d post-treatment.
[c]Not available as mortality was already 100% or not scored.

4 h. Mean survival times (86.7–94.1 h) for these *C. hemipterus* strains were significantly longer ($P < 0.05$, Mantel–Cox log rank test) than that of the Monheim susceptible (MY) strain (Table 4). Mean survival time of two strains (Queensland and Krystal Point) could not be generated as there were no mortality even up to 4 d posttreatment.

Table 5. Mean survival times and mortality of *C. lectularius* in ActiveGuard mattress liner 6-hour exposure experiment

Species Strain (country)	*n*	Mean survival time (h)	Std. error	95% CI	Cumulative % mortality 1-d	Cumulative % mortality 4-d
C. lectularius						
Harlan susceptible (US)	50	1.8	0.1	1.5–2.1	100	–[a]
Epic Center (US)	50	86.7*	4.3	78.2–95.2	–[a]	12
Richmond (US)	50	68.4*	6.1	56.6–80.3	–[a]	64
Vinton (US)	50	96.0*	0	96.0–96.0	–[a]	2

*Indicates that the mean survival time was significantly different from that of the Harlan susceptible (US) strain (Mantel–Cox log-rank test, $P < 0.05$).
[a]Not available as mortality was already 100% or not scored.

In the US trials, all Harlan strain bed bugs were knocked down within 6 h of being exposed to the mattress liner (Table 5). In contrast, bed bug mortality for the Vinton, Epic Center, and Richmond strains was low even at 4 d postexposure to 6 h contact with the Mattress liner, with 2%, 12%, and 64% mortality, respectively. The mean survival times for all field strains were significantly longer ($P < 0.05$, Mantel–Cox log-rank test) than that of the Harlan susceptible strain (Table 5). Control mortalities ranged from 0 to 2.5% at 4 d posttreatment.

Double Fabric Experiment

The Australian trials registered 82% mortality with the susceptible Monheim bed bugs at 7 d posttreatment. With the least resistant strain, Ripponlea, mortality was only 46% at 7 d (Table 6). Due to the poor performance of the Mattress liner in the previous trials, the double fabric study was not conducted using the other modern field strains. Control mortalities for the Australian trials were 0–5% at 7 d posttreatment.

The adult bed bugs for all modern field-collected *C. hemipterus* strains exhibited 0–16% mortality after 96 h of exposure in the Malaysian trials. Mean survival times for Bukit Mertajam, Madam Mo, and Tanjung Tokong strains were significantly longer ($P < 0.05$; Mantel–Cox log-rank test) than that of the Monheim susceptible (MY) strain. The mean survival times for Queesland (AUS), Kuala Lumpur (MY), Saujana (MY), and Krystal Point (MY) strains could not be generated as there were very low mortalities (0–6%) at 4 d posttreatment (Table 6). There were no control mortalities at 4 d posttreatment.

The susceptible Monheim bed bugs in the UK trials experienced 58% mortality after a 21 d exposure period (Table 6). The F4 and London N7 strains reported no mortality, whereas only 2% and 28% mortalities were observed in L1 and Malmö strains, respectively, after 21 d of direct exposure to the untreated fabric, covering the Mattress liner. Control mortalities for the UK trial ranged from 0 to 2.5% at 21 d posttreatment.

Discussion

The allethrin-mat bioassay developed by Dang et al. (2015a) was conducted to determine the presence and level of pyrethroid resistance in all test bed bug strains before being subjected to the performance evaluations for the ActiveGuard mattress liner. According to Dang et al. (2015a), the mat assay is suitable for pyrethroid resistance profiling due to its cost-effectiveness, its speed, and the low number of insects required (10 or more bed bugs). It also helped standardize the tests conducted in multiple laboratories by minimizing any discrepancies that could arise from having to formulate solutions or using different treatment surfaces. Based on Dang et al. (2015a), a bed bug strain is considered pyrethroid-resistant when less than 100% mortality or knockdown is observed after an hour of exposure to the d-allethrin mat. The investigations herein found that all modern field-collected strains showed considerably less than 100% mortality after being exposed to the mat for 1 h, indicating all modern field strains possessed some level of resistance against pyrethroids.

Insecticide resistance tests using the mat assay for the Australian modern field strains determine the following resistance rankings (from the least to most resistant): Adelaide, Ripponlea, Parramatta, Tamworth, Sydenham, Newcastle, Alice Springs, and Melbourne. These results using the allethrin-based assay closely correlate with the testing used by Lilly et al. (2018) when evaluating bed bug response to a discriminating dose of another pyrethroid, deltamethrin. The mat assay results for the Malaysian strains also conformed to the results of Leong et al. (2020b) when compared with other pyrethroids. These authors reported that all modern field-collected *C. hemipterus* strains in their study were resistant to the pyrethroid-based liquid formulations, Pesguard FG161 (Sumitomo, USA, containing cyphenothrin and tetramethrin) and Sumithrin (Sumitomo, USA, containing phenothrin). Hence, the parallels in efficacy with previously published research between the different pyrethroids have demonstrated the utility of the mat assay.

The mattress liner failed to achieve appreciable levels of mortality against most of the strains of *C. lectularius* and *C. hemipterus* used in this study. In fact, for many strains, in spite of being in constant contact with the liner for 7 d or more, no to low mortalities were observed. Its efficacy was further reduced when bed bugs were held on the mattress liner for a short period of either 4 or 6 h. This test was intended to evaluate the liner efficacy if bed bugs move from one place to another, rather than stay in continuous contact with the one surface. Furthermore, a reduction in liner efficacy was documented when a piece of fabric was placed over the liner. For example, the mean survival times for the susceptible Monheim (AUS and UK) strains were 7,117 times and 180 times greater, respectively, in the double-sheet fabric experiment compared to the continuous exposure experiment (Tables 6 and 3). This experiment simulated a real-world situation, whereby the directions for use of the product state that a 'mattress pad, barrier encasement or bottom sheet [is fitted] over the mattress liner' so that the impregnated liner is not in direct contact with human skin (ActiveGuard, 2012).

The results from the Malaysian assays are the first report on the performance of a pyrethroid-based mattress liner for control of *C. hemipterus*. As noted above, the previous research from the Malaysian laboratory observed poor bed bug control efficacy when

Table 6. Mean survival times and mortality of *C. lectularius* and *C. hemipterus* in continuous ActiveGuard mattress liner double-fabric exposure experiment

Species Strain (country)	*n*	Mean survival time (h)[a]	Std. error	95% CI	Cumulative % mortality 1-d	4-d	7-d	21-d
C. lectularius								
Monheim susceptible (MY)	50	3.2	0.3	2.7–3.7	100	–[c]	–[c]	–[c]
Monheim susceptible (AUS)	50	133.4	4.1	125.5–141.4	0	18	82	–
Monheim susceptible (UK)	50	360.5	20.8	319.8–401.2	0	–	28	58
Ripponlea (AUS)	50	136.9*	7.7	121.8–152.1	12	26	46	–
F4 (UK)	50	504.0*	0	504.0–504.0	0	–[c]	0	0
L1 susceptible (UK)	50	436.8*	16.5	404.5–469.1	0	–[c]	12	28
London N7 (UK)	50	–[b]			0	–[c]	0	0
Malmö (Sweden)	50	504.0*	0	504.0–504.0	0	–[c]	0	2
C. hemipterus								
Queensland (AUS)	50	–[b]			0	0	–[c]	–[c]
Kuala Lumpur (MY)	50	–[b]			0	0	–[c]	–[c]
Bukit Mertajam (MY)	50	95.0*	0.8	93.4–96.6	0	16	–[c]	–[c]
Saujana (MY)	50	–[b]			0	6	–[c]	–[c]
Krystal Point (MY)	50	–[b]			0	0	–[c]	–[c]
Madam Mo (MY)	50	89.5*	2.8	83.9–95.1	4	16	–[c]	–[c]
Tanjung Tokong (MY)	50	92.6*	2.0	88.7–96.6	0	10	–[c]	–[c]

An * indicates that the strain's mean survival time is significantly different from that of the corresponding susceptible strain (Mantel–Cox log-rank test, $P < 0.05$).
[b]Analysis not possible due to no mortality even at 4 or 21 d post-treatment.
[c]Not available as mortality was already 100% or not scored.

pyrethroid formulations, including Pesguard FG161 and sumithrin (Leong et al. 2020a) were evaluated. The authors suggested that pyrethroids were not suitable in the management of *C. hemipterus*. Thus, the poor performance of the permethrin-impregnated mattress liner was not unexpected.

In this study, the Madam Mo strain displayed very high resistance to d-allethrin in the 'mat assay' but showed moderate resistance to permethrin on the mattress liner. On the contrary, the Kuala Lumpur strain that registered a lower resistance level on the 'mat assay' was showing 'very high' resistance to the permethrin-impregnated mattress liner. Such discrepancy has been observed in the German cockroach, *Blatella germanica* L., especially when the insecticide resistant strains have P450 monooxygenase and/or elevated esterases as resistance mechanisms (Chai and Lee 2010). Different isozymes may contribute varying efficiencies towards detoxification of different pyrethroids. Furthermore, although evaluations on mat and mattress liner are both surface-contact assays, the manner on how the pyrethroids are being impregnated and formulated in the two products differ.

The only strains whereby a moderate degree of knockdown or mortality was achieved using the mattress liner were the Ripponlea (Australia), Richmond (US), and L1 (UK) strains. Of the modern field strains tested for resistance to date, the Ripponlea strain is one of the least resistant bed bugs maintained in colony in Australia (Lilly et al. 2018). The US Richmond strain has been held in the laboratory since 2008 and is likely to have lost much of its resistance, as it is known that resistance confers a significant fitness cost in *C. lectularius* (Gordon et al. 2015). This strain is known to possess only the one *kdr* mutation (haplotype B) and presumably less resistant than those with multiple mutations (Zhu et al. 2010). However, even when tested against these three strains, the mattress liner failed to kill all the bed bugs. The L1 strain was colonized c.1970 and is a known susceptible strain, having overlapping 95% fiducial limits with the KT_{50} in the mat assay, with the Monheim control strain, even though the Resistance Ratio suggested it had 'Low' resistance.

A concerning aspect of this study is a sheer number of surviving bed bugs in all the trials. Even with 7 d continual exposure, no knockdown or mortality occurred in multiple strains. The use of a permethrin-impregnated mattress liner over the short or long-term is likely to select for the more pyrethroid-resistant bed bug individuals within a population and drive pyrethroid resistance levels even higher. Ultimately, this will make bed bug control more challenging, and may also lead to cross-resistance with other chemical classes, as has recently been reported (Romero and Anderson 2016, Leong et al. 2020a).

It was observed that in the continuous exposure and double-fabric experiments, the Monheim susceptible strain displayed LT values and mean survival times that are significantly different between the laboratories. What is particularly confounding is that the Malaysian stock was originally derived from the Australian culture back in 2014. The major difference in colony maintenance is the blood source, with the Malaysian laboratory using freshly drawn rabbit blood in lithium heparin tubes. It is unknown if this could have had an effect in reducing the susceptibility of the bed bugs, however Aak and Rukke (2013) reported that 1% heparinized rat blood reduced egg numbers and body size of bed bugs compared to those that were rodent-fed. It is also possible that a change in blood sources could potentially alter the gut symbiont diversity, which subsequently led to changes in susceptibility. There may also be subtle but inapparent differences in the assay methodology and mortality readings by the experimenters that could have contributed to the differences.

Caveats of the study are that there were some differences between observation intervals and minor variations in bioassay procedures between the laboratories. The differences in the bioassay intervals are accounted for by the statistical analyses and the minor differences in experimental procedures do not affect the validity of the conclusions drawn from the work.

Recently it was reported that the ActiveGuard mattress liner has been effective at preventing the reintroduction of bed bugs in

affordable housing (Gooch 2021). Population genetics analysis of bed bug infestations within multi-unit dwellings has demonstrated that disparate infestations in the same building have a high degree of relatedness (Booth et al. 2012). These authors suggested that most infestations start within a building with a single female and that active, and human-mediated, dispersal leads the initial infestation to spread throughout the building. This information indicates that new bed bug introductions are likely to be a relatively rare event, with the implication that if the infestation is eliminated, then the property should remain bed bug free for an extended period. An example of this was with a staff accommodation complex on the campus of a major healthcare facility in Australia. Bed bugs (*C. lectularius*) were first reported in 2003, and due to poor management, subsequently spread to 20% of the 333 rooms in the building (Doggett and Russell 2008). Consequently, every room was inspected for bed bugs, and all active infestations eliminated. Since then, no new infestations have been reported (Doggett 2018). Hence, if a product claims to prevent bed bugs, and none ensue, it is not possible to determine if the lack of insects is due to the efficacy of the product, or the fact that none have been reintroduced.

The present study demonstrated that permethrin-impregnated mattress liners have very little efficacy when it comes to controlling modern field strains of *C. lectularius* and *C. hemipterus* from different parts of the world. Almost all the strains tested showed moderate to very high levels of pyrethroid resistance. In light of the poor efficacy for killing these modern field strains in the laboratory experiments and the wide-spread pyrethroid resistance in modern field populations of both common and tropical bed bugs, we anticipate that the use of the pyrethroid-based mattress liners in the field will have limited efficacy in controlling or preventing bed bug infestations. Therefore, permethrin-impregnated mattress liners should not be recommended as part of an overall bed bug management program.

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