Journal of Economic Entomology, 110(2), 2017, 558–566
doi: 10.1093/jee/tow296
Advance Access Publication Date: 23 January 2017
Research article

Effects of Different Surfaces and Insecticide Carriers on Residual Insecticide Bioassays Against Bed Bugs, *Cimex* spp. (Hemiptera: Cimicidae)

Kai Dang,[1] G. Veera Singham,[2] Stephen L. Doggett,[3] David G. Lilly,[4] and Chow-Yang Lee[1,5]

[1]Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia (kaidangusm@gmail.com; chowyang@usm.my), [2]Centre for Chemical Biology, Universiti Sains Malaysia, 10 Persiaran Bukit Jambul, Penang 11900, Malaysia (veerasingham@usm.my), [3]Department of Medical Entomology, Pathology West, Westmead Hospital, Westmead, NSW 2145, Australia (Stephen.Doggett@health.nsw.gov.au), [4]Department of Medical Entomology, University of Sydney, Westmead Hospital, Westmead, NSW 2145, Australia (dlil7680@uni.sydney.edu.au), and [5]Corresponding author, e-mail: chowyang@usm.my

Subject Editor: Michael Rust

Received 7 September 2016; Editorial decision 26 November 2016

Abstract

The performance of five insecticides (bendiocarb, deltamethrin, DDT, malathion, and imidacloprid) using three application methods (oil-based insecticide films on filter paper, and acetone-based insecticide deposits on two substrates: filter paper and glass) was assessed against a susceptible strain of *Cimex lectularius* (L.) and two resistant strains of *Cimex hemipterus* (F.). Substrate type significantly affected ($P < 0.05$) the insecticide knockdown response of the susceptible strain in acetone-based insecticide bioassays, with longer survival time on filter paper than on the glass surface. With the exception of deltamethrin, the different diluents (oil and acetone) also significantly affected ($P < 0.05$) the insecticide knockdown response of the susceptible strain in the filter paper-based insecticide bioassays, with longer survival time with acetone as the diluent. For both strains of *C. hemipterus*, there were no significant effects with the different surfaces and diluents for all insecticides except for malathion and imidacloprid, which was largely due to high levels of resistance. The lower effectiveness for the insecticide acetone-based treatment on filter paper may be due to crystal bloom. This occurs when an insecticide, dissolved in a volatile solvent, is applied onto absorptive surfaces. The effect is reduced on nonabsorptive surfaces and slowed down with oil-based insecticides, whereby the oil forms a film on absorptive surfaces. These findings suggest that nonabsorptive surfaces should be used in bioassays to monitor insecticide resistance. If absorptive surfaces are used in bioassays for testing active ingredients, then oil-based insecticides should be preferably used.

Key words: Bed bug, comparison, bioassay, insecticide resistance, neonicotinoid

The common bed bug *Cimex lectularius* L. and the tropical bed bug *Cimex hemipterus* (F.) (Hemiptera: Cimicidae) are cryptic, nocturnal, hematophagous ectoparasites that have been ubiquitous and annoying pests for much of human history (Usinger 1966, Potter 2011, Koganemaru and Miller 2013). The development of synthetic organic insecticides provided an efficient and economical method of controlling insect pests, including bed bugs (Potter 2011). Subsequently, bed bugs gradually became uncommon, especially in economically developed countries, during the latter half of the 20th century (Potter 2011, Davies et al. 2012, Doggett et al. 2012). However, over the past 15–20 yr, bed bugs of both species have made a global resurgence (Potter 2006, Davies et al. 2012, Doggett et al. 2012, Koganemaru and Miller 2013, Alalawi 2015, Dang et al. 2015c). Insecticide resistance has been postulated as a significant contributor to the resurgence of bed bugs (Romero et al. 2007).

Evaluation of insecticide formulations and detection of insecticide resistance are prerequisite steps for ensuring that the most appropriate insecticides and products are used in bed bug eradication. Bioassays are routinely conducted to evaluate insecticide formulations and detect insecticide resistance in various insect pests, including bed bugs (Busvine 1958; WHO 1992; Fletcher and Axtell 1993; Myamba et al. 2002; Boase et al. 2006; Moore and Miller 2006, 2009; Karunaratne et al. 2007; Romero et al. 2007, 2009, 2010; Barile et al. 2008; Steelman et al. 2008; Yoon et al. 2008; Kweka et al. 2009; Wang et al. 2009; Zhu et al. 2010, 2013; Kilpinen et al. 2011, Adelman et al. 2011; Tawatsin et al. 2011; Mamidala et al. 2012; Koganemaru et al. 2013; Choe and Campbell 2014; Dang

© The Authors 2017. Published by Oxford University Press on behalf of Entomological Society of America.
All rights reserved. For Permissions, please email: journals.permissions@oup.com

et al. 2015a,b,c; Lilly et al. 2015, 2016a,b; Romero and Anderson 2016). Biochemical and molecular assays also are used to monitor the underlying resistance mechanisms in bed bugs (Karunaratne et al. 2007; Yoon et al. 2008; Zhu et al. 2010, 2012, 2013; Seong et al. 2010; Adelman et al. 2011; Bai et al. 2011; Mamidala et al. 2012; Koganemaru et al. 2013; Dang et al. 2015b, 2015c; Palenchar et al. 2015; Romero and Anderson 2016).

The most common bioassay method used to test the effectiveness of residual insecticides is to apply insecticide films or deposits on surfaces of various substrates such as filter paper, cloth, glass plates, vials, plywood, wooden or metal plates, and other substrates (Nagasawa 1959). Such substrates can generally be divided into absorptive and nonabsorptive types. Of the absorptive surfaces, filter paper is the most often used in bioassays for bed bugs (WHO 1992; Fletcher and Axtell 1993; Myamba et al. 2002; Boase et al. 2006; Karunaratne et al. 2007; Romero et al. 2007, 2009, 2010; Barile et al. 2008; Yoon et al. 2008; Kweka et al. 2009; Wang et al. 2009; Seong et al. 2010; Zhu et al. 2010, 2013; Tawatsin et al. 2011; Durand et al. 2012; Mamidala et al. 2012). Other absorptive surfaces such as hardboard panels (Moore and Miller 2006, Polanco et al. 2011), mosquito mats (Dang et al. 2015a, 2015b, 2015c), wood (Choe and Campbell 2014), polyester netting (Myamba et al. 2002), pine plywood, cardboard, cotton cloth, and polyester (Fletcher and Axtell 1993) have also been used in assays against bed bugs. Nonabsorptive surfaces used in bed bug residual bioassays include glass vials (Steelman et al. 2008, Hardstone et al. 2015), glass plates (Kilpinen et al. 2011), ceramic tiles (How and Lee 2011), and metal (Fletcher and Axtell 1993).

Whether in the form of technical grade or in formulated products, insecticides can be introduced onto the treatment surfaces using different diluents, including acetone and oil. However, formulated products are normally tested with the recommended label diluent, commonly tap water, so as to reflect field efficacy. In the World Health Organization (WHO) susceptibility test kits (WHO 1992, 2013), insecticides are dissolved in a mixture of acetone and different oil types (e.g., DDT [organochlorines {OCs}] in Risella oil, malathion [organophosphates {OPs}] and bendiocarb [carbamates] in olive oil, and pyrethroids in silicone oil) prior to impregnation of the insecticide on the filter paper. These kits are widely used against various insect pests, including bed bugs, for the monitoring of insecticide resistance (WHO 1963, 1970, 1976, 1992; Karunaratne et al. 2007; Tawatsin et al. 2011). Over the past two decades, many researchers dissolve technical-grade insecticides in acetone only for the preparation of deposits on filter paper for bed bug bioassays (Fletcher and Axtell 1993; Boase et al. 2006; Romero et al. 2007; Barile et al. 2008; Yoon et al. 2008; Kweka et al. 2009; Seong et al. 2010; Zhu et al. 2010, 2013; Mamidala et al. 2012). In addition, water-based deposits have been used to evaluate insecticide products and insecticide resistance in bed bugs (Fletcher and Axtell 1993, Moore and Miller 2006, Kilpinen et al. 2011, Polanco et al. 2011, Tawatsin et al. 2011).

To date, few reports have compared the performance of insecticides on different substrates (absorptive vs. nonabsorptive surfaces) and in different diluents (such as oil and acetone, which produce oil films and dry deposits, respectively). In this study, we compared the performance of five classes of insecticides (e.g., OCs [DDT], OPs [malathion], carbamates [bendiocarb], pyrethroids [deltamethrin], and neonicotinoids [imidacloprid]) using three application methods (acetone-based insecticide deposits on filter paper and glass petri dishes, and oil-based insecticide films on filter paper) against *C. lectularius* and *C. hemipterus* to determine how these parameters affect insecticide resistance assays. Results of this study can be used to improve current bioassay methods for monitoring insecticide resistance in bed bugs.

Materials and Methods

Bed Bug Populations

A laboratory insecticide-susceptible strain of *C. lectularius* (Monheim) and two field-collected insecticide-resistant strains of *C. hemipterus* (KL-MY and QLD-AU; see Dang et al. 2015c) were used in this study. All strains were reared in the Urban Entomology Laboratory, School of Biological Sciences, Universiti Sains Malaysia at 25 ± 2°C, 75 ± 10% RH, and a photoperiod of 10 h. Once a week, all bed bugs were offered a bloodmeal on either a human volunteer or freshly drawn rabbit blood mixed with 1% heparin using the Hemotek membrane feeding system (Discovery Workshops, Accrington, UK). No insecticide selection was done on these strains. The Monheim *C. lectularius* strain was used as an insecticide-susceptible strain, as no standard susceptible strain of *C. hemipterus* could be sourced worldwide.

Chemicals

Five technical-grade insecticides, namely, DDT (98%, WHO, Geneva, Switzerland), malathion (92.8%, PESTANAL, Sigma Aldrich Laborachemikalien GmBh, Munich, Germany), bendiocarb (90.5%, Fisons Incorporated, Bedford, MA, USA), deltamethrin (97%, Bayer Environmental Science, Kuala Lumpur, Malaysia), and imidacloprid (95%, Bayer CropScience, Hawthorn East, Victoria, Australia), were used in this study. The following types of oil were used as diluents: Risella oil (Shell Malaysia Limited, Kuala Lumpur, Malaysia), olive oil (91.6%, Pomace Olive Oil Cosmetic Grade, Parchem Trading Ltd., New Rochelle, NY, USA, CAS NO. 68956-68-3, EINECS No. 273-313-5), and silicone oil (Dow Corning 556 Cosmetic Grade Fluid, Dow Corning Thailand Limited, Bangkok, Thailand). Acetone was bought from R & M Marketing (Essex, UK, CAS NO. 67-64-1).

Bioassay Procedure

Three methods were used in this study: 1) acetone-diluted insecticide deposits on filter paper (abbreviated as PAPER), 2) acetone-based insecticide deposits on a glass petri dish (GLASS), and 3) oil-based insecticide films on filter paper (OIL/PAPER). In the first treatment, the filter papers (diameter 55 mm, Filtres Fioroni, Ingre, France, Reference No.: 0601A00003) were impregnated with 0.3 ml of acetone-diluted insecticides, with acetone alone as the control. In the second treatment, 0.8 ml of acetone-diluted insecticide was added to a glass petri dish (90 mm diameter × 18 mm height). The petri dish was rotated by hand to ensure an even and complete insecticide application. The acetone was then left to completely evaporate, leaving a uniformly applied insecticide residue on the surface. The control glass petri dish was treated with an equal volume of acetone only. In the third treatment, a 0.3 ml solution consisting of a mixture of acetone-diluted insecticide and oil (Risella oil for DDT; olive oil for malathion and bendiocarb; silicone oil for deltamethrin and imidacloprid) at a ratio of 1 to 2 (insecticide was dissolved in one part of acetone first, and then diluted by two part of oil) was used to impregnate the filter paper. For the control, the filter paper was impregnated with an equal volume of acetone/oil mixture at the same ratio. All impregnated papers and glass petri dishes were left in a fume hood for air drying over 24 h prior to the bioassay. The malathion comes in a technical-grade liquid form. When the

acetone-diluted malathion was treated with the glass petri dishes, the insecticide (entirely liquid form of malathion) was visibly left on the petri dish after the acetone had completely evaporated. Table 1 shows the discriminating concentration of each insecticide used in the bioassays.

Bed bugs were fed to repletion 5 d before testing. For each replicate, 10 adult bed bugs of randomly mixed sex and age were transferred from rearing jars into a plastic petri dish (60 mm diameter × 15 mm height, Citotest Labware Manufacturing Co. Ltd., Jiangsu, China, CAS NO. 33100601) lined with the filter paper treated by insecticide or into a glass petri dish coated with insecticide. The cumulative number of insects knocked down was recorded at regular time intervals for continuous exposure up to 72 h (bendiocarb, deltamethrin, and malathion) and 120 h (DDT and imidacloprid). For the control, 10 insects were exposed to the control filter paper and the control glass petri dish. An insect was considered to be knocked down if it was unable to move or right itself when gently touched. A minimum of three replicates was used for each insecticide in each treatment, and for the controls.

Statistical Analysis

Control knockdown was corrected using Abbott's (1925) formula. Data were pooled and subjected to probit analysis (Finney 1971). The goodness-of-fit test was used to confirm that the data set met the assumptions of the probit model. KT_{50} values, that is, time to knockdown 50% of tested insects exposed to a certain concentration of insecticide, were considered significantly different ($P < 0.05$) when their 95% confidence intervals (CIs) did not overlap (Payton et al. 2003, Wheeler et al. 2006). The survival time data in different treatments were analyzed using survival analysis (log-rank test). The distribution of survival time was described using the survivorship function S (t). Kaplan–Meier survival curves were generated. Significance was set at $P < 0.05$. All statistical analyses were performed using SPSS v22 for Windows (IBM Corp., Armonk, NY, USA).

Results

Insecticide Efficacy Assay

With the exception of DDT and imidacloprid, all other compounds tested against the Monheim strain of *C. lectularius* showed high levels of insecticidal efficacy in the PAPER, GLASS, and OIL/PAPER treatments (Table 2). However, there are different insecticidal efficacies detected against the susceptible Monheim strain when the insecticides were deposited on different test surfaces and dissolved in different diluents on filter papers (Fig. 1A–J). Insects from the susceptible strain were completely knocked down within 30 min by bendiocarb (i.e., 30 min [PAPER], 15 min [GLASS], and 20 min [OIL/PAPER]) and deltamethrin (i.e., 30 min [PAPER], 20 min [GLASS], and 30 min [OIL/PAPER]), and within 2.5 h by malathion (i.e., 2.5 h [PAPER], 65 min [GLASS], and 1.5 h [OIL/PAPER]). For DDT and imidacloprid, insects from the susceptible strain were all knocked down by DDT within 72 h (PAPER), 24 h (GLASS), and 12 h (OIL/PAPER) and by imidacloprid within a maximum of 4 h in the GLASS (3 h) and OIL/PAPER (4 h) treatments. However, only 95% of the insects were knocked down even after 120 h of exposure in the imidacloprid PAPER treatment (Table 2).

Both the QLD-AU and KL-MY strains of *C. hemipterus* exhibited high levels of resistance to bendiocarb, DDT, and deltamethrin, compared with the Monheim *C. lectularius* strain. After 72 h of exposure, up to 13.3% (PAPER: 10 ± 5.8%, GLASS: 13.3 ± 6.7%, OIL/PAPER: 13.3 ± 8.8%) and 30% (PAPER: 3.3 ± 3.3%, GLASS: 16.7 ± 8.8%, OIL/PAPER: 30 ± 25.2%) of the QLD-AU and KL-MY strains, respectively, were knocked down by deltamethrin, while the values were at most 60% (PAPER: 30 ± 10%, GLASS: 60 ± 5.8%, OIL/PAPER: 26.7 ± 8.8%) and 16.7% (PAPER: 16.7 ± 6.7%, GLASS: 13.3 ± 3.3%, OIL/PAPER: 13.3 ± 8.8%) for bendiocarb, respectively (Table 2). DDT caused a maximum knockdown of 40% (PAPER: 40 ± 10%, GLASS: 10 ± 5.8%, OIL/PAPER: 26.7 ± 6.7%) and 46.7% (PAPER: 20 ± 15.3%, GLASS: 16.7 ± 8.8%, OIL/PAPER: 46.7 ± 12%) for both QLD-AU and KL-MY strains, respectively, at 120 h of exposure (Table 2). The QLD-AU strain showed moderate levels of resistance to malathion (Table 2), compared with the susceptible Monheim strain, with complete

Table 1. Discriminating concentrations used in this study

Insecticides	Susceptibility baseline	Discriminating concentration [f]	Carriers[g]			References
			PAPER	GLASS	OIL/PAPER	
Bendiocarb[a]	38.2 mg AI m^{-2}	382 mg AI m^{-2}	Acetone	Acetone	Olive oil	Barile et al. (2008)
Deltamethrin[b]	19.2 mg AI m^{-2}	192 mg AI m^{-2}	Acetone	Acetone	Silicone oil	Barile et al. (2008)
Imidacloprid[c]	NA	192 mg AI m^{-2}[h]	Acetone	Acetone	Silicone oil	NA
DDT[d]	NA	2% (2525 mg AI m^{-2})[i]	Acetone	Acetone	Risella oil	WHO (1992)
Malathion[e]	NA	5% (6313 mg AI m^{-2})[i]	Acetone	Acetone	Olive oil	WHO (1992)

[a] Carbamate.
[b] Pyrethroids
[c] Neonicotinoids
[d] Organochlorines
[e] Organophosphates.
[f] The baseline for each insecticide (bendiocarb, deltamethrin) was multiplied by 10 and served as the discriminating concentration used for bioassays. For DDT and malathion, twice the concentration/dosage that killed 100% of the susceptible insect strain served as the discriminating concentration (WHO 1992).
[g] PAPER and GLASS, insecticide was dissolved in acetone only; OIL/PAPER, insecticide was dissolved in acetone first and then mixed with different types of oil. The ratio of acetone and oil was 1 to 2 (insecticides were dissolved by one part of acetone first, and then diluted by two part of oil). The type of oil for diluting each insecticide follows WHO (2013).
[h] As the susceptibility to imidacloprid is similar to that of pyrethroids in pyrethroid-susceptible *C. lectularius* strains identified by Steelman et al. (2008), in this study, the susceptibility baseline to deltamethrin was multiplied by 10 to serve as the discriminating concentration of imidacloprid for the bioassay.
[i] The application rates of DDT and malathion, respectively, used in this study.
AI = active ingredient.

Table 2. KT_{50}s and KT_{95}s of bed bugs (*Cimex* spp.) exposed to the insecticides for different bioassay treatments

Strains	Insecticides	N	Method	KT_{50}* (95% CI) (min)	KT_{95} (95% CI) (min)	χ^2 (df)	Slop	Knockdown (%) (exposure time)
Monheim	Deltamethrin	30	PAPER	19.2 (18.0–20.1)	26.0 (24.3–29.2)	3.23 (3)	5.46 ± 0.90	100 (30 min)
		30	GLASS	13.5 (12.8–14.2)	18.4 (17.1–20.5)	1.78 (3)	5.35 ± 0.72	100 (20 min)
		30	OIL/PAPER	19.4 (18.4–20.2)	25.2 (23.8–27.9)	3.18 (3)	6.30 ± 0.98	100 (30 min)
	DDT	30	PAPER	1338.2 (1112.9–1556.3)	3449.8 (2732.0–5198.5)	0.07 (2)	1.74 ± 0.28	100 (72 h)
		30	GLASS	1027.0 (1002.1–1054.2)	1218.7 (1164.0–1319.1)	2.69 (3)	9.61 ± 1.43	100 (24 h)
		30	OIL/PAPER	244.8 (220.6–266.6)	533.4 (451.1–710.8)	3.12 (6)	2.11 ± 0.31	100 (12 h)
	Malathion	30	PAPER	84.1 (79.2–88.3)	121.483 (110.75–144.46)	0.64 (3)	4.47 ± 0.75	100 (2.5 h)
		30	GLASS	39.3 (37.3–41.1)	56.0 (52.1–62.4)	0.68(5)	4.65 ± 0.57	100 (65 min)
		30	OIL/PAPER	44.7 (42.6–46.9)	65.6 (60.3–74.7)	2.40 (5)	4.29 ± 0.52	100 (1.5 h)
	Bendiocarb	30	PAPER	18.9 (17.7–19.9)	26.6 (24.7–29.82)	2.15 (3)	4.82 ± 0.68	100 (30 min)
		30	GLASS	10.1 (9.4–10.7)	14.3 (13.1–16.6)	1.57 (2)	4.72 ± 0.70	100 (15 min)
		30	OIL/PAPER	12.2 (11.4–13.1)	19.9 (17.9–23.3)	2.47 (4)	3.40 ± 0.42	100 (20 min)
	Imidacloprid	120	PAPER	3960.8 (3022.7–3213.3)	6599.4 (5493.5–10214.2)	4.48 (2)	3.22 ± 0.24	95 ± 2.6 (120 h)
		30	GLASS	94.8 (90.9–98.7)	130.3 (122.0–143.6)	1.10 (5)	5.19 ± 0.61	100 (3 h)
		30	OIL/PAPER	121.1 (110.1–130.8)	204.1 (181.7–247.3)	0.72 (3)	3.15 ± 0.46	100 (4 h)
QLD-AU	Deltamethrin	30	PAPER	>4,320	>4,320	NA	NA	10 ± 5.8 (72 h)
		30	GLASS	>4,320	>4,320	NA	NA	13.3 ± 6.7 (72 h)
		30	OIL/PAPER	>4,320	>4,320	NA	NA	13.3 ± 8.8 (72 h)
	DDT 2%	30	PAPER	>7,200	>7,200	NA	NA	40 ± 10 (120 h)
		30	GLASS	>7,200	>7,200	NA	NA	10 ± 5.8 (120 h)
		30	OIL/PAPER	>7,200	>7,200	NA	NA	26.7 ± 6.7 (120 h)
	Malathion	30	PAPER	913.4 (797.4–1021.8)	1898.1 (1536.4–3002.9)	0.11 (2)	2.25 ± 0.45	100 (36 h)
		30	GLASS	574.8 (510.8–654.7)	1485.3 (1179.8–2130.7)	1.67 (5)	1.73 ± 0.21	100 (30 h)
		30	OIL/PAPER	1028.9 (912.0–1141.9)	1921.5 (1631.4–2577.7)	1.37 (2)	2.63 ± 0.44	100 (36 h)
	Bendiocarb	30	PAPER	>4,320	>4,320	NA	NA	30 ± 10 (72 h)
		30	GLASS	3391.8 (2645.5–5652.6)	>4,320	0.12 (3)	0.91 ± 0.23	60 ± 5.8 (72 h)
		30	OIL/PAPER	>4,320	>4,310	NA	NA	26.7 ± 8.8 (72 h)
	Imidacloprid	90	PAPER	>7,200	>7,200	NA	NA	41.1 ± 4.2 (120 h)
		30	GLASS	69.2 (64.5–73.9)	125.0 (111.0–149.5)	1.42 (6)	2.78 ± 0.32	100 (2.5 h)
		30	OIL/PAPER	80.5 (73.1–87.5)	157.4 (134.3–208.4)	6.24 (4)	2.45 ± 0.38	100 (3 h)
KL-MY	Deltamethrin	30	PAPER	>4,320	>4,320	NA	NA	3.3 ± 3.3 (72 h)
		30	GLASS	>4,320	>4,320	NA	NA	16.7 ± 8.8 (72 h)
		30	OIL/PAPER	>4,320	>4,320	NA	NA	30 ± 25.2 (72 h)
	DDT	30	PAPER	>7,200	>7,200	NA	NA	20 ± 15.3 (120 h)
		30	GLASS	>7,200	>7,200	NA	NA	16.7 ± 8.8 (120 h)
		30	OIL/PAPER	>7,200	>7,200	NA	NA	46.7 ± 12 (120 h)
	Malathion	30	PAPER	>4,320	>4,320	NA	NA	43.3 ± 8.8 (72 h)
		30	GLASS	722.9(619.5–848.4)	2834.0 (2115.6–4391.6)	0.93 (6)	1.20 ± 0.14	96.7 ± 3.3 (72h)
		30	OIL/PAPER	1982.9 (1521.3–2629.2)	>4,320	1.14 (2)	0.95 ± 0.19	76.7 ± 8.8 (72 h)
	Bendiocarb	30	PAPER	>4,320	>4,320	NA	NA	16.7 ± 6.7 (72 h)
		30	GLASS	>4,320	>4,320	NA	NA	13.3±3.3 (72 h)
		30	OIL/PAPER	>4,320	>4,320	NA	NA	13.3 ± 8.8 (72 h)
	Imidacloprid	90	PAPER	>7,200	>7,200	NA	NA	0 (120 h)
		30	GLASS	69.3 (60.5–75.6)	133.1 (115.5–174.4)	2.06 (4)	2.52 ± 0.44	100 (3 h)
		30	OIL/PAPER	117.0 (109.1–126.8)	224.3 (190.7–292.7)	4.82 (6)	2.53 ± 0.33	100 (4 h)

*KT_{50}s whose 95% CIs do not overlap are significantly different ($P < 0.05$).
NA = not applicable.

knockdown by 36 h of exposure in the PAPER and OIL/PAPER treatments and by 30 h in the GLASS treatment. However, the KL-MY strain had high levels of resistance to malathion, with 43.3, 96.7, and 76.7% of insects knocked down in the PAPER, GLASS, and OIL/PAPER treatments, respectively, after 72 h of exposure.

Both the resistant QLD-AU and KL-MY strains were susceptible to imidacloprid in the GLASS and OIL/PAPER treatments, compared with the susceptible Monheim strain. The QLD-AU strain was entirely knocked down within 3 h by imidacloprid in the GLASS (2.5 h) and OIL/PAPER (3 h) treatments, while the KL-MY strain was completely knocked down within 4 h (e.g., GLASS [3 h] and OIL/PAPER [4 h]; Table 2). However, these two resistant strains showed significantly reduced susceptibility to imidacloprid in the PAPER treatment (Table 2), with the 120 h mean cumulative knockdown percentage of the QLD-AU at 41.1 ± 4.2%, and of the KL-MY strain at 0%.

Performance of Insecticide Deposits on Different Surfaces

The performance of all insecticides against *C. lectularius* was significantly different ($P < 0.05$) between the PAPER and GLASS treatments (different test surfaces; Table 2). The survival time of the susceptible insects was significantly longer in the PAPER treatment than that in the GLASS treatment for all insecticides (Fig. 1A, C, E, G, and I).

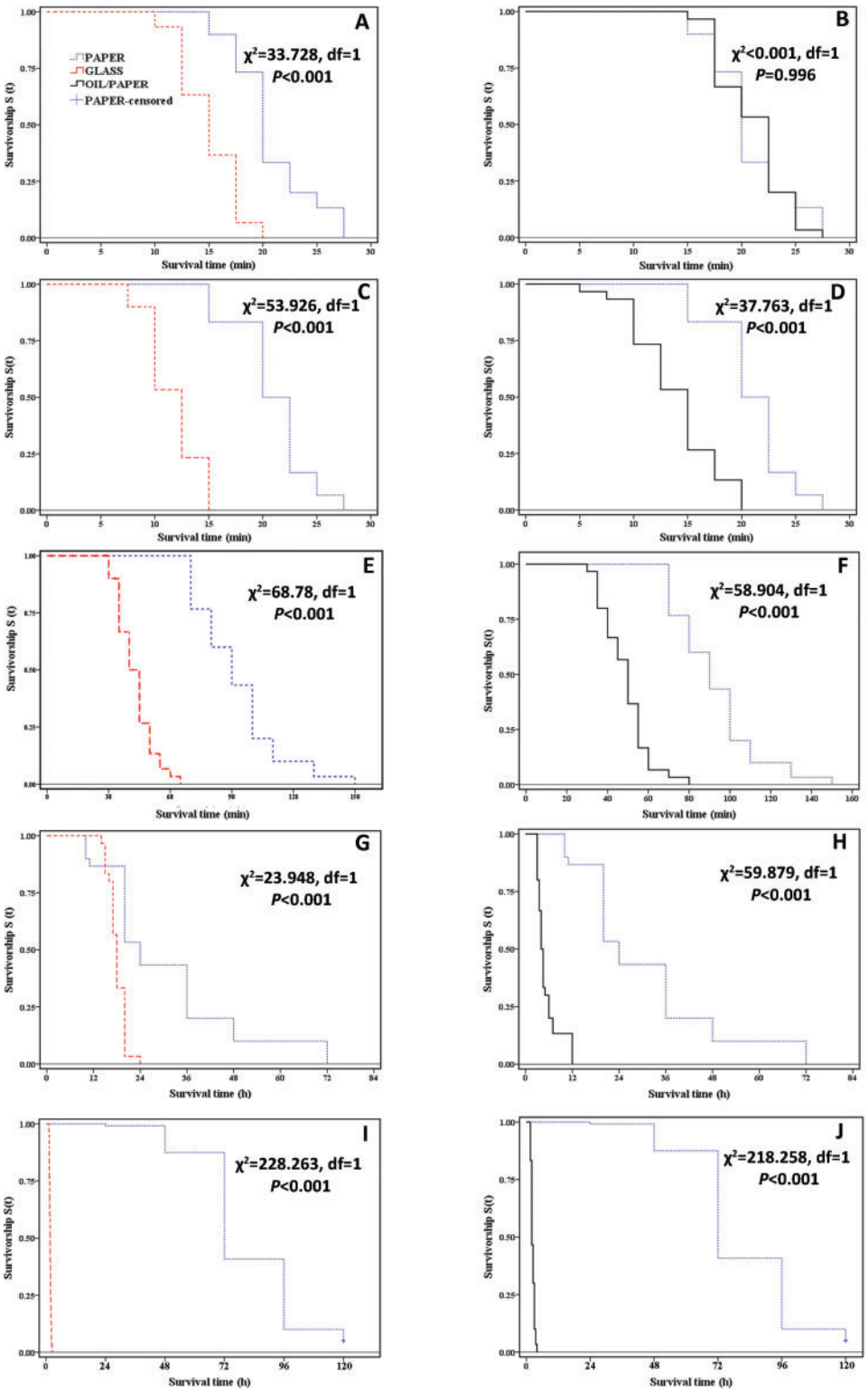

Fig. 1. Kaplan–Meier survival analyses for the susceptible Monheim strain of *C. lectularius* when exposed to acetone-based deposits of all five insecticides (**A**–and **B**: deltamethrin, **C** and **D**: bendiocarb, **E** and **F**: malathion, **G** and **H**: DDT, **I** and **J**: imidacloprid) on filter paper (PAPER) and glass petri dishes (GLASS), and to the oil-based films of the insecticides on filter paper (OIL/PAPER). **A**, **C**, **E**, **G**, **I**: comparisons of survival time between the PAPER and GLASS treatments; **B**, **D**, **F**, **H**, **J**: comparisons of survival time between the PAPER and OIL/PAPER treatments. Survivorship function S (t) was defined as the probability that an individual survives longer than time "t" (Online figure in color).

The performance of imidacloprid against QLD-AU and KL-MY strains showed significant differences between the PAPER and GLASS treatments (Table 2). The survival time of both resistant strains exposed to imidacloprid in the GLASS treatment was significantly shorter than that in the PAPER treatment (Fig. 2A and C). Similarly, the performance of malathion against QLD-AU and

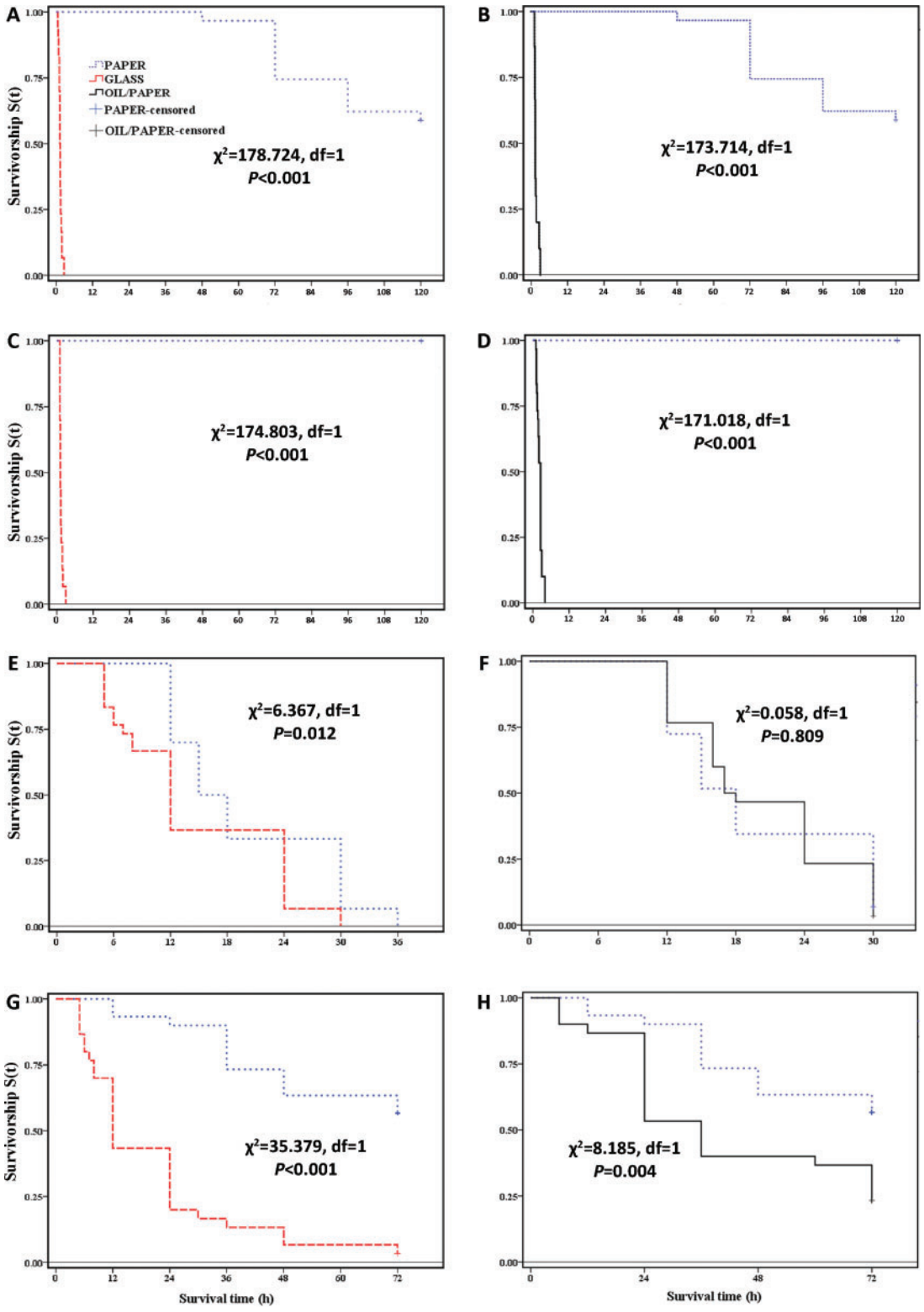

Fig. 2. Kaplan–Meier survival analyses for the resistant QLD-AU (**A** and **B**, **E** and **F**) and KL-MY (**C** and **D**, **G** and **H**) strains of *C. hemipterus* when exposed to acetone-based deposits of imidacloprid (**A**–**D**) and malathion (**E**–**H**) on filter paper (PAPER) and glass petri dishes (GLASS), and to the oil-based films of these two insecticides on filter paper (OIL/PAPER). **A**, **C**, **E**, **G**: comparisons of survival time between the PAPER and GLASS treatments; **B**, **D**, **F**, **H**: comparisons of survival time between the PAPER and OIL/PAPER treatments. Survivorship function S (t) was defined as the probability that an individual survives longer than time "t" (Online figure in color).

KL-MY strains showed significant differences between the PAPER and GLASS treatments (Table 2; Fig. 2E and G). However, performance of bendiocarb, deltamethrin, and DDT against the KL-MY and QLD-AU strains showed no significant differences among the different surfaces largely due to high levels of resistance (Table 2).

Performance of Insecticides in Different Carriers

With the exception of deltamethrin, the performance of all insecticides against the susceptible *C. lectularius* strain differed significantly between the PAPER and OIL/PAPER treatments (Table 2; Fig. 1B, D, F, H, and J). The insecticides, especially DDT and imidacloprid, in the PAPER treatment showed significantly low levels of efficacy when compared with that observed in the OIL/PAPER treatment (Table 2). The survival time of the susceptible insects exposed to DDT and imidacloprid in the PAPER treatment was significantly longer than that observed in the OIL/PAPER treatment (Fig. 1H and J).

The performance of imidacloprid differed significantly between the PAPER and OIL/PAPER treatments in both *C. hemipterus* strains (Table 2), with significantly reduced susceptibility in the PAPER treatment compared with that of the OIL/PAPER treatment (Fig. 2B and D). The performance of malathion against QLD-AU strain did not significantly differ between the two carriers on filter paper (Table 2; Fig. 2F). For the KL-MY strain, performance of malathion differed significantly between the PAPER and OIL/PAPER treatments (Table 2), with much longer survival time in the PAPER treatment (Fig. 2H). The performance of bendiocarb, deltamethrin, and DDT in different carriers was not significantly different for the KL-MY and QLD-AU strains, judging from the high levels of resistance (Table 2).

Discussion

During the past two decades, bed bugs have undergone a major resurgence worldwide, and insecticide resistance appears to be a significant factor responsible for their comeback (Romero et al. 2007). The bed bug resurgence has led to widespread research on insecticide efficacy, insecticide resistance, resistance mechanisms, and management strategies. Bioassay, especially the use of the residual surface method, is the most common method for evaluating insecticide formulations and insecticide resistance in bed bugs. The current study demonstrated that knockdown responses of bed bugs differed significantly when the same insecticide was deposited on different test surfaces and when the insecticide was carried by different diluents, even on the same test surface. For example, the Monheim strain of *C. lectularius* exposed to the acetone-based insecticides on glass petri dishes survived for a significantly shorter time than when exposed to the insecticides placed on filter paper. In addition, the Monheim strain exposed to insecticides (excluding deltamethrin) carried by oil survived for a significantly shorter time than when carried by acetone only in the filter paper treatment. These findings suggest that the appropriate method of exposure in bioassays is critical for accurately evaluating insecticide products and insecticide resistance in bed bugs (Fletcher and Axtell 1993).

In this study, acetone-diluted DDT deposits on filter paper showed significantly slower efficacy to the susceptible Monheim strain, with all bed bugs knocked down by 72 h of exposure. Conversely, acetone-diluted DDT deposits on glass petri dishes and oil-diluted DDT films on filter paper exhibited significantly higher efficacy, with all insects knocked down by 24 h and 12 h, respectively. A similar phenomenon for DDT residue tests was found in previous studies against *C. lectularius* (Busvine and Barnes 1947). This was also observed for imidacloprid in this study against both *C. lectularius* and *C. hemipterus* (Table 2) and for fenvalerate (pyrethroids) in another previous study against *C. lectularius* (Fletcher and Axtell 1993).

The physical condition of an insecticide residue governs its toxicity, as its structure determines how much of the insecticide is available to an insect moving over the treated surface (Lyon 1965). For insecticide that comes in a technical-grade crystal form, the insecticide dissolved in volatile solvents applied to an absorbent surface (e.g., filter paper) can result in a crystal bloom (a phenomenon of insecticide crystallization induced by the absorptive surface) on the surface after evaporation (Lyon 1965). Furthermore, DDT on compressed fiber board showed a greater extent of crystallization than other insecticides (Barlow and Hadaway 1952a). Busvine and Barnes (1947) reported that the volatile solvents in acetone-diluted DDT impregnated onto filter paper underwent evaporation, leading to irregular size and distribution of DDT crystals with consequent erratic test results. Barlow and Hadaway (1952b) found that the rate of action of DDT particles (the discrete microfragments of a deposit, whether crystalline or noncrystalline, and amorphous in nature) picked up by mosquitoes increased as the size decreased. Therefore, the crystal bloom may have increased the size of DDT particles on the filter paper, resulting in a significantly slower efficacy of DDT against the Monheim strain. Although DDT can crystallize on the glass petri dishes (nonabsorptive surface) after the volatile solvent has evaporated, the phenomenon of crystal bloom may not occur, or occur to a lesser extent, compared with that on the filter paper (absorptive surface). This phenomenon may also explain why the acetone-diluted imidacloprid deposits on filter paper showed a significantly slower efficacy against the susceptible Monheim strain, and both the resistant QLD-AU and KL-MY strains, compared with that on glass petri dishes.

The use of nonabsorptive surfaces (e.g., glass petri dishes, glass vials, glass plates, glass bottles, metal, jars, and tiles) may avoid or reduce crystal bloom of insecticides induced by absorptive surfaces. Fletcher and Axtell (1993) found that deposits of five of six tested insecticide products on a nonabsorptive metal surface showed higher efficacy against one laboratory strain of *C. lectularius* than insecticides on absorptive surfaces (e.g., pine plywood, cardboard, cotton cloth, and polyester). In this study, the efficacies of all acetone-diluted insecticides on glass petri dishes to the Monheim strain were significantly greater than that of all acetone-diluted insecticides on filter paper. Due to the reason of the nonabsorptive surface resulting in less crystal bloom, glass jars are often used as the treatment surface in bioassays for various insect pests, such as cockroaches (WHO 2002) and mosquitoes (Brogdon and Chan 2010, Owusu et al. 2015).

Furthermore, nonabsorptive surfaces can prevent the insecticide (if the insecticide comes in a technical-grade liquid form) from absorbing through the surface. As observed in this study, malathion was entirely visible and all left on the glass petri dish (nonabsorptive surface) after the diluent acetone (volatile solvent) was completely evaporated. However, the insecticide absorbed into the filter paper in the other tests. Insects can more easily pick up the insecticide on the nonabsorptive surface, compared with that on the absorptive surface. This explains why the insects (both susceptible *C. lectularius* and resistant *C. hemipterus*) survived significantly shorter on malathion in the GLASS treatment than that in the PAPER treatment.

Oil-based insecticide films on absorptive surfaces (e.g., filter papers) also may undergo slow or reduced crystallization of insecticides (Barlow and Hadaway 1952a). In fact, the nonvolatile oil solution maintains an oil film that prevents or slows down the rapid crystallization of insecticides, resulting in prolonged effectiveness of the insecticide deposits (Barlow and Hadaway 1952a). Busvine and Nash (1953) reported that filter papers in residual bioassays should

be impregnated with standard oil solution of insecticides. The WHO standardized this method for the preparation of test kits for the detection and measurement of resistance in adult mosquitos (Busvine 1958). Currently, the WHO standard susceptibility test kits (WHO 2013) are widely used to detect insecticide resistance in a range of insect pests (Owusu et al. 2015), including bed bugs (WHO 1963, 1970, 1976, 1992; Karunaratne et al. 2007, Tawatsin et al. 2011). The results of the current study demonstrate that the oil-based insecticides (excluding deltamethrin), especially DDT and imidacloprid, on filter paper had significantly higher efficacy against bed bugs compared with the acetone-based deposits on filter paper.

Based on the findings herein, we suggest that nonabsorptive surfaces (e.g., glass petri dishes, glass vials, and tile) should be used in bioassays when evaluating bed bug insecticide resistance. However, in natural infestations, bed bugs tend to congregate more frequently on rough absorptive surfaces rather than smooth nonabsorptive substrates (Doggett 2013). Thus, in order to obtain a more accurate estimate of potential field efficacy with formulated insecticide products, absorptive surfaces should be used as the substrate of choice. For this reason, absorptive surfaces such as filter paper are commonly used in bioassays for bed bugs (Myamba et al. 2002; Boase et al. 2006; Moore and Miller 2006, 2009; Karunaratne et al 2007, Romero et al. 2007, 2009, 2010; Barile et al 2008; Kweka et al. 2009; Zhu et al. 2010, 2013; Tawatsin et al. 2011).

If absorptive surfaces were to be used in residual bioassays for resistance determination, then the insecticide should be mixed in an oil carrier before impregnation of the surface. The use of acetone-based (volatile solvent-based) technical-grade insecticide deposits on filter paper for insecticide resistance assays should be avoided.

Acknowledgments

We thank Mr. Adanan Che Rus (Vector Control Research Unit, Universiti Sains Malaysia) for providing the different oil types used in this study, Universiti Sains Malaysia for supporting the post-doctoral fellowship of K.D., and 1001/PCCB/811340 Universiti Sains Malaysia RU Grant and Semco Co. Ltd (Japan) for financial support.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Adelman, Z. N., K. A. Kilcullen, R. Koganemaru, M. A. E. Anderson, T. D. Anderson, and D. M. Miller. 2011. Deep sequencing of pyrethroid-resistant bed bugs reveals multiple mechanisms of resistance within a single population. PLoS ONE 6: e26228.

Alalawi, A. H. 2015. Bed bugs epidemic in the United States. Entomol. Ornithol. Herpetol. 4: 143.

Bai, X., P. Mamidala, S. P. Rajarapu, S. C. Jones, and O. Mittapalli. 2011. Transcriptomics of the bed bug (*Cimex lectularius*). PLoS ONE 6: e16336.

Barile, J., R. Naven, G. Nentwig, R. Pospischil, and B. Reid. 2008. Laboratory and field evaluation of deltamethrin and bendicarb to control *Cimex lectularius* (Heteroptera: Cimicidae), pp. 105–111. *In* W. H. Robinson and D. Bajomi (eds.), Proceedings of the Sixth International Conference on Urban Pests. Budapest, Hungary, 13 to 16 July 2008. OOK-Press, Pápai, Hungary.

Barlow, F., and A. B. Hadaway. 1952a. Some factors affecting the availability of contact insecticides. Bull. Entomol. Res. 43: 91–100.

Barlow, F., and A. B. Hadaway. 1952b. Studies on aqueous suspensions of insecticides. Part II. Quantitative determinations of weights of DDT picked up and retained. Bull. Entomol. Res. 42: 769–777.

Boase, C., G. Small, and R. Naylor. 2006. Interim report on insecticide susceptibility status of UK bedbugs. Prof. Pest Control. 6–7.

Brogdon, W., and A. Chan. 2010. Guidelines for evaluating insecticide resistance in vectors using the CDC bottle bioassay/methods in Anopheles research, 2nd edn. CDC technical report, Centers for Disease Control and Prevention, Atlanta, GA.

Busvine, J. R. 1958. Insecticide-resistance in bed-bugs. Bull. World Health Org. 19: 1041–1052.

Busvine, J. R., and S. Barnes. 1947. Observations on mortality among insects exposed to dry insecticidal films. Bull. Entomol. Res. 38: 81–90.

Busvine, J. R., and R. Nash. 1953. The potency and persistence of some new synthetic insecticides. Bull. Entomol. Res. 44: 371–376.

Choe, D., and K. Campbell. 2014. Effect of feeding status on mortality response of adult bed bugs (Hemiptera: Cimicidae) to some insecticide products. J. Econ. Entomol. 107: 1206–1215.

Dang, K., D. G. Lilly, W. Bu, and S. L. Doggett. 2015a. Simple, rapid and cost-effective technique for the detection of pyrethroid resistance in bed bugs, *Cimex* spp. (Hemiptera: Cimicidae). Austral Entomol. 54: 191–196.

Dang, K., C. S. Toi, D. G. Lilly, W. Bu, and S. L. Doggett. 2015b. Detection of knockdown resistance mutations in the common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), in Australia. Pest Manag. Sci. 71: 914–922.

Dang, K., C. S. Toi, D. G. Lilly, C. Y. Lee, R. Naylor, A. Tawatsin, U. Thavara, W. Bu, and S. L. Doggett. 2015c. Identification of putative *kdr* mutations in the tropical bed bug, *Cimex hemipterus* (Hemiptera: Cimicidae). Pest Manag. Sci. 71: 1015–1020.

Davies, T.G.E., L. M. Field, and M. S. Williamson. 2012. The re-emergence of the bed bug as a nuisance pest: Implications of resistance to the pyrethroid insecticides. Med. Vet. Entomol. 26: 241–254.

Doggett, S. L., D. E. Dwyer, P. F. Peñas, and R. C. Russell. 2012. Bed bugs: Clinical relevance and control options. Clin. Microbiol. Rev. 25: 164–192.

Doggett, S. L. 2013. Do you have bed bugs? A help guide for the identification of bed bug infestations. Doggett, S. L., Sydney, Australia

Durand, R., A. Cannet, Z. Berdjane, C. Bruel, D. Haouchine, P. Delaunay, and A. Izri. 2012. Infestation by pyrethroids resistant bed bugs in the suburb of Paris, France. Parasite 9: 381–387.

Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge University Press, London.

Fletcher, M. G., and R. C. Axtell. 1993. Susceptibility of the bedbug, *Cimex lectularius*, to selected insecticides and various treated surfaces. Med. Vet. Entomol. 7: 69–72.

Hardstone, M. C., J. P. Strycharz, J. Kim, I. Park, K. S. Yoon, Y. J. Ahn, L. C. Harrington, S. H. Lee, and J. M. Clark. 2015. Development of multifunctional metabolic synergists to suppress the evolution of resistance against pyrethroids in insects that blood feed on humans. Pest. Manag. Sci. 71: 842–849.

How, Y. F., and C. Y. Lee. 2011. Surface contact toxicity and synergism of several insecticides against different stages of the tropical bed bug, *Cimex hemipterus* (Hemiptera: Cimicidae). Pest Manag. Sci. 67: 734–740.

Karunaratne, S.H.P.P., B. T. Damayanthi, M.H.J. Fareena, V. Imbuldeniya, and J. Hemingway. 2007. Insecticide resistance in the tropical bedbug *Cimex hemipterus*. Pestic. Biochem. Phys. 88: 102–107.

Kilpinen, O., M. Kristensen, and K.M.V. Jensen. 2011. Resistance differences between chlorpyrifos and synthetic pyrethroids in *Cimex lectularius* population from Denmark. Parasitol. Res. 109: 1461–1464.

Koganemaru, R., and D. M. Miller. 2013. The bed bug problem: Past, present, and future control methods. Pestic. Biochem. Phys. 106: 177–189.

Koganemaru, R., D. M. Miller, and Z. N. Adelman. 2013. Robust cuticular penetration resistance in the common bed bug (*Cimex lectularius* L.) correlates with increased steady-state transcript levels of CPR-type cuticle protein genes. Pestic. Biochem. Phys. 106: 190–197.

Kweka, E. J., B. J. Mwang'onde, E. E. Kimaro, S. Msangi, F. Tenu, and A. M. Mahande. 2009. Insecticides susceptibility status of the bedbugs (*Cimex lectularius*) in a rural area of Magugu, Northern Tanzania. J. Glob. Infect. Dis. 1: 102–106.

Lilly, D. G., M. P. Zalucki, C. Orton, R. C. Russell, C. E. Webb, and S. L. Doggett. 2015. Confirmation of insecticide resistance in *Cimex lectularius* Linnaeus (Hemiptera: Cimicidae) in Australia. Austral. Entomol. 54: 96–99.

Lilly, D. G., K. Dang, C. E. Webb, and S. L. Doggett. 2016a. Evidence for metabolic pyrethroid resistance in the common bed bug (Hemiptera: Cimicidae). J. Econ. Entomol. 109: 1364–1368.

Lilly, D. G., S. L. Latham, C. E. Webb, and S. L. Doggett. 2016b. Cuticle thickening in a pyrethroid-resistant strain of the common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae). PLoS ONE 11: e0153302.

Lyon, R. L. 1965. Structure and toxicity of insecticide deposits for control of bark beetles. Tech. Bull. 1343: 1–54.

Mamidala, P., A. J. Wijeratne, S. Wijeratne, K. Kornacker, B. Sudhamalla, L. J. Rivera-Vega, A. Hoelmer, T. Meulia, S. C. Jones, and O. Mittapalli. 2012. RNA-Seq and molecular docking reveal multi-level pesticide resistance in the bed bug. BMC Genome 13: 6.

Moore, D. J., and D. M. Miller. 2006. Laboratory evaluations of insecticide product efficacy for control of *Cimex lectularius*. J. Econ. Entomol. 99: 2080–2086.

Moore, D. J., and D. M. Miller. 2009. Field evaluations of insecticide treatment regimens for control of the common bed bug, *Cimex lectularius* (L.). Pest Manag. Sci. 65: 332–338.

Myamba, J., C. A. Maxwel, A. Asidi, and C. F. Curtis. 2002. Pyrethroid resistance in tropical bed bugs, *Cimex hemipterus*, associated with use of treated bednets. Med. Vet. Entomol. 16: 448–451.

Nagasawa, S. 1959. Biological assay of insecticide residues. Annu. Rev. Entomol. 4: 319–342.

Owusu, H. F., D. Jančáryová, D. Malone, and P. Müller. 2015. Comparability between insecticide resistance bioassays for mosquito vectors: Time to review current methodology? Parasite Vector 8: 357.

Palenchar, D. J., K. J. Gellatly, K. S. Yoon, K. Y. Mumcuoglu, U. Shalom, and J. M. Clark. 2015. Quantitative sequencing for the determination of *kdr*-type resistance allele (V419L, L925I, I936F) frequencies in common bed bug (Hemiptera: Cimicidae) populations collected from Israel. J. Med. Entomol. 52: 1018–1027.

Payton, M. E., M. H. Greenstone, and N. Schenker. 2003. Overlapping confidence intervals or standard error intervals: What do they mean in terms of statistical significance? J. Insect Sci. 3: 34.

Polanco, A. M., D. M. Miller, and C. C. Brewster. 2011. Survivorship during starvation for *Cimex lectularius* L. Insects 2: 232–242.

Potter, M. F. 2006. The perfect storm: An extension view on bed bugs. Am. Entomol. 52: 102–104.

Potter, M. F. 2011. The history of bed bug management – with lessons from the past. Am. Entomol. 57: 14–25.

Romero, A., M. F. Potter, D. A. Potter, and K. F. Haynes. 2007. Insecticide resistance in the bed bug: A factor in the pest's sudden resurgence? J. Med. Entomol. 44: 175–178.

Romero, A., M. F. Potter, and K. F. Haynes. 2009. Evaluation of piperonyl butoxide as a deltamethrin synergist for pyrethroid-resistant bed bugs. J. Econ. Entomol. 102: 2310–2315.

Romero, A., M. F. Potter, and K. F. Haynes. 2010. Evaluation of chlorfenapyr for control of the bed bug, *Cimex lectularius* L. Pest Manag. Sci. 66: 1243–1248.

Romero, A., and T. D. Anderson. 2016. High levels of resistance in the common Bed Bug, *Cimex lectularius* (Hemiptera: Cimicidae), to neonicotinoid Insecticides. J. Med. Entomol. 53: 727–731.

Seong, K. M., D. Y. Lee, K. S. Yoon, D. H. Kwon, H. C. Kim, T. A. Klein, J. M. Clark, and S. H. Lee. 2010. Establishment of quantitative sequencing and filter contact vial bioassay for monitoring pyrethroid resistance in the common bed bug, *Cimex lectularius*. J. Med. Entomol. 47: 592–599.

Steelman, C. D., A. L. Szalanski, R. Trout, J. A. McKern, C. Solorzano, and J. W. Austin. 2008. Susceptibility of the bed bug *Cimex lectularius* L. (Heteroptera: Cimicidae) collected in poultry production facilities to selected insecticides. J. Agric. Urban Entomol. 25: 41–51.

Tawatsin, A., U. Thavara, J. Chompoosri, Y. Phusup, N. Jonjang, and C. Khumsawads. 2011. Insecticide resistance in bedbugs in Thailand and laboratory evaluation of insecticides for the control of *Cimex hemipterus* and *Cimex lectularius* (Hemiptera: Cimicidae).J. Med. Entomol. 48: 1023–1030.

Usinger, R. L. 1966. Monograph of Cimicidae (Hemiptera-Heteroptera), Thomas Say Foundation, Vol. 7. Entomological Society of America, College Park, MD.

Wang, C., T. Gibb, and G. W. Bennett. 2009. Evaluation of two least toxic integrated pest management programs for managing bed bugs (Heteroptera: Cimicidae) with discussion of a bed bug intercepting device. J. Med. Entomol. 46: 566–571.

Wheeler, M. W., R. M. Park, and A. J. Barler. 2006. Comparing median lethal concentration values using confidence interval overlap or ratio tests. Environ. Toxicol. Chem. 25: 1441–1444.

(WHO) World Health Organization. 1963. Insecticide resistance and vector control. Thirteenth report of the WHO expert committee on insecticides. WHO Technical Report Series No. 265. World Health Organization, Geneva, Switzerland.

(WHO) World Health Organization. 1970. Insecticide resistance and vector control. Seventeenth report of the WHO expert committee on insecticides. WHO Technical Report Series No. 433. World Health Organization, Geneva, Switzerland.

(WHO) World Health Organization. 1976. Resistance of vectors and reservoirs of disease to pesticides. Twenty-second report of the WHO expert committee on insecticides. WHO Technical Report Series No. 585. World Health Organization, Geneva, Switzerland.

(WHO) World Health Organization. 1992. Vector resistance to Pesticides. Fifteenth report of the WHO Expert Committee on Vector Biology and Control. World Health Organization, Geneva, Switzerland.

(WHO) World Health Organization. 2002. Supplies for monitoring insecticide resistance in disease vectors. World Health Organization, Geneva, Switzerland.

(WHO) World Health Organization. 2013. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. World Health Organization, Geneva, Switzerland.

Yoon, K. S., D. H. Kwon, J. P. Strycharz, C. S. Hollingsworth, S. H. Lee, and J. M. Clark. 2008. Biochemical and molecular analysis of deltamethrin resistance in the common bed bug (Hemiptera: Cimicidae). J. Med. Entomol. 45: 1092–1101.

Zhu, F., J. Wigginton, A. Romero, A. Moore, K. Ferguson, R. Palli, M. F. Potter, K. F. Haynes, and S. R. Palli. 2010. Widespread distribution of knockdown resistance mutations in the bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), populations in the United States. Arch. Insect Biochem. Phys. 73: 245–257.

Zhu, F., S. Sams, T. Moural, K. F. Haynes, M. F. Potter, and S. R. Palli. 2012. RNA interference of NADPH-cytochrome P450 reductase results in reduced insecticide resistance in the bed bug, *Cimex lectularius*. PLoS ONE 7: e31037.

Zhu, F., H. Gujar, J. R. Gordon, K. F. Haynes, M. F. Potter, and S. R. Palli. 2013. Bed bugs evolved unique adaptive strategy to resist pyrethroid insecticides. Sci. Rep. 3: 1456.