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Effect of *Wolbachia* on insecticide susceptibility in lines of *Aedes aegypti*

N.M. Endersby* and A.A. Hoffmann

Department of Genetics, Bio21 Institute, The University of Melbourne, Victoria 3010, Australia

Abstract

Two stable infections of *Wolbachia pipientis*, *w*MelPop and *w*Mel, now established in *Aedes aegypti*, are being used in a biocontrol program to suppress the transmission of dengue. Any effects of *Wolbachia* infection on insecticide resistance of mosquitoes may undermine the success of this program. Bioassays of *Ae. aegypti* were conducted to test for differences in response to insecticides between *Wolbachia* infected (*w*MelPop, *w*Mel) and uninfected lines. Insecticides screened were bifenthrin, the pyrethroid commonly used for adult knockdown, as well as larvicides: *Bacillus thuringiensis* var. *israelensis*, the organophosphate, temephos and the insect growth regulator, s-methoprene. While differences in response between lines were detected for some insecticides, no obvious or consistent effects related to presence of *Wolbachia* infection were observed. Spreading *Wolbachia* infections are, therefore, unlikely to affect the efficacy of traditional chemical control of mosquito outbreaks.

Keywords: *Aedes aegypt*, *Wolbachia*, insecticide resistance, dengue

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Introduction

Strains of the bacterium, *Wolbachia pipientis*, have been introduced into natural populations of the dengue vector, *Aedes aegypti*, in order to reduce transmission of dengue virus (Hoffmann *et al*., 2011; Walker *et al*., 2011). The effects of the *Wolbachia* infections on aspects of mosquito fitness, such as longevity, fecundity, egg desiccation resistance, locomotor ability and blood feeding, have been documented (Evans *et al*., 2009; Turley *et al*., 2009; Yeap *et al*., 2011). Susceptibility to insecticides is an important trait which also needs to be understood in this context, particularly because chemicals are used routinely to reduce mosquito populations in areas of dengue infection and research goes on to improve applications and formulations (Oki *et al*., 2011; Paz-Soldan *et al*., 2011; Harburguer *et al*., 2012). The possibility of *Wolbachia* infections influencing pesticide resistance was raised by the observation that there was a higher *Wolbachia* load in insecticide resistant *Culex pipiens* compared with insecticide susceptible individuals, possibly due to decreased control of *Wolbachia* load as a physiological cost of insecticide resistance (Berticat *et al*., 2002; Duron *et al*., 2006). Similar results have been shown with another symbiont, R*ickettsia*, which increased susceptibility of *Bemisia tabaci* to some groups of insecticides, both singly and as a double infection with a second symbiont (Ghanim & Kontsedalov, 2009).

The stable infections of *Wolbachia* which have been introduced to *Ae. aegypti* are the *w*MelPop and *w*Mel strains (Hoffmann *et al*., 2011; Walker *et al*., 2011). The *w*MelPop strain has a higher density of infection than the *w*Mel strain, and the former infection is more widespread throughout the mosquito body and has greater negative effects on fitness (Walker *et al*., 2011). Duron *et al*. (2006) showed that fitness cost of a *Wolbachia* infection increases with *Wolbachia* density in *Cx. pipiens*. Therefore, it is possible that mosquitoes containing *Wolbachia* could have different susceptibilities to the same insecticide if *Wolbachia* has any effect on this trait.

Insecticides for mosquito control cover a variety of modes of action and target different life stages. We looked at resistance to four different insecticides in this study. Bifenthrin, a pyrethroid, is a knockdown insecticide that targets adult mosquitoes. The bacterial insecticide, *Bacillus thuringiensis* var. *israelensis*, trade name VectoBac® WG, is toxic through ingestion and so targets the larval stage. Temephos is an organophosphate insecticide and is widely

*Author for correspondence
Fax: +61 3 8344 2279
E-mail: nancye@unimelb.edu.au

used as a mosquito larvicide throughout the world, particularly for control of *Ae. aegypti*. In Australia, temephos is sold in a slow-release granular formulation with trade name Abate™. Finally, s-methoprene is a juvenile hormone mimic sold as Prolink™ Liquid Larvicide. This insecticide kills mosquitoes as they undergo metamorphosis from fourth instar to pupa or at eclosion. The study undertook bioassays of *Ae. aegypti* to test for differences in response to insecticides between *Wolbachia* infected (*w*MelPop, *w*Mel) and uninfected lines.

Methods

Mosquito lines

Lines of *Ae. aegypti* containing either the *w*MelPop ('popcorn') or *w*Mel strain of *Wolbachia pipientis* were generated as described by Yeap *et al.* (2011) (*w*MelPop) and Walker *et al.* (2011) (*w*Mel) and were then maintained in discrete generations as mass bred populations (designated by MB in line name). The populations used in this study and their designation are given in table 1. Populations had been backcrossed for a minimum of four generations to a wild type Cairns strain of *Ae. aegypti* to ensure a homogeneous genetic background and were maintained at an adult size of several hundred mosquitoes to minimize any drift effects between infected and uninfected populations prior to testing for resistance (see Yeap *et al.*, 2011). *Wolbachia* infection status of the lines was confirmed through a Q-PCR assay described by Lee *et al.* (2012). Larvae were maintained on TetraMin® Tropical Fish Food tablets Rich Mix (from here onwards referred to as TetraMin®) and adults were fed on human arms as described by Yeap *et al.* (2011).

Bifenthrin (adults)

Glass vials were coated with formulated bifenthrin (Talstar® 250EC: bifenthrin 250 g l^{-1}) (table 2) at a range of concentrations. Test insects were females less than 72 h old without blood feeding. Insects were exposed to insecticide in a treated vial for 1 h and then transferred to a clean vial. They were fed on 10% sucrose solution in cotton wool in the vial lid before and after exposure. Knockdown was scored at 1 h and 24 h using the absence of flight as the knockdown criterion, although this also includes mosquitoes that had died. Temperature throughout the bioassay was 26.5 ± 1°C (no light). Data were subjected to probit analysis using PoloPlus Version 2 (© 2002–2011 LeOra Software, Petaluma, CA, USA).

Three comparisons involving *w*MelPop and two comparisons involving *w*Mel were carried out. In the first, backcross populations (i.e. *w*MelPop on field background) AOC2 and BUC2 were compared with the non-*Wolbachia* population C2MB from which they were derived by backcrossing. The second comparison involved C67 (field origin), O1MB (*w*MelPop line), AOMB (*w*MelPop on field background) and BOMB (*w*MelPop on field background). The third comparison involved C67 (field origin), O1MB (*w*MelPop line), U3MB (*w*MelPop line), AOMB (*w*MelPop on field background) and BOMB (*w*MelPop on field background).

In the first *w*Mel comparison, C89 mosquitoes (uninfected, field origin) were compared with mosquitoes from the *w*Mel (original strain) and *w*C89G1 (*w*Mel backcrossed on field background) strains. This comparison was then repeated.

Table 1. Lines of *Aedes aegypti* (Wolbachia-infected and uninfected) used in insecticide bioassays.

Mosquito line	*Wolbachia* infection status	Genetic background
C2MB	Uninfected	Wildtype collected in Cairns 2009–2010 Field origin
C67	Uninfected	Field origin
C89	Uninfected	Field origin
O1MB	*w*MelPop	*w*MelPop inbred laboratory line
U3MB	*w*MelPop	*w*MelPop inbred laboratory line
AOC2*	*w*MelPop	*w*MelPop on field background (C2MB)
BUC2**	*w*MelPop	*w*MelPop on field background (C2MB)
AOMB*	*w*MelPop	*w*MelPop on field background (C2MB)
BOMB**	*w*MelPop	*w*MelPop on field background (C2MB)
*w*Mel	*w*Mel	*w*Mel inbred laboratory line
*w*C45	*w*Mel	*w*Mel on field background (C45)
*w*C89	*w*Mel	*w*Mel on field background (C89)

* Crossed according to Scheme A; **crossed according to Scheme B of Yeap *et al.* (2011)

Bacillus thuringiensis *(larvae)*

Eggs of *Ae. aegypti* laid on sandpaper were hatched by submersion in 3 l of reverse osmosis (RO) water containing one tablet of TetraMin®. Trays of larvae were maintained at 26°C, 12L:12D. Two days after hatching, the density of larvae was reduced to 200 larvae per 4 l containing one tablet of TetraMin® in a new tray. Three to four days after hatching, larvae were collected with an eyedropper for use in the following bioassays:

Two experiments with slightly different procedures were undertaken to compare the strains. In the first, reverse osmosis (RO) water (5 ml) was dispensed into 30-ml centrifuge tubes (BD Falcon™). Four days after hatching, larvae of a standard size were collected with a dropper, taking a minimum amount of water. Five larvae were placed into each tube and the volume was made up to 10 ml with RO water; 20 ml of *Bacillus thuringiensis* (VectoBac® WG) solution (table 1) was dispensed into each Falcon™ tube using a 10-ml pipette to make the range of concentrations: 0, 0.008, 0.016, 0.019, 0.024, 0.026, 0.027, 0.028, 0.029 ppm). Eight replicates of each concentration were made. Lids were placed on tubes. Mortality was scored at 48 h. Temperature throughout the bioassay was 26.5 ± 1°C (no light). Lines tested were C45 (field origin); *w*Mel; U3MB, O1MB (*w*MelPop laboratory lines); AOMB, BOC2 (*w*MelPop outbred lines) and *w*C45 (*w*Mel on field background).

In the second experiment, reverse osmosis (RO) water (190 ml) was dispensed into 250-ml glass jars (Cospak Pty Ltd, Minto, NSW, Australia). Three days after hatching, larvae of a standard size were collected with a dropper and placed in small medicine cups (Huhtamaki Australia Pty Ltd, Rhodes, NSW, Australia). Eight to 14 larvae were transferred from the medicine cups into each jar using a tea strainer; 10 ml of *Bacillus thuringiensis* (VectoBac® WG) solution to make final concentrations of 0, 0.008, 0.012, 0.016, 0.019 and 0.025 ppm

Table 2. Insecticides used to bioassay lines of *Aedes aegypti*.

Insecticide	Active ingredient	Product name	Company	Concentration range for bioassay (ppm)
bifenthrin	250 g l^{-1}	Talstar® 250EC Insecticide/Miticide	FMC Australasia Pty Ltd	0.010–5.000
Bacillus thuringiensis subsp. *israelensis* serotype H14	3000 ITU mg^{-1}	VectoBac® WG	Valent BioSciences	*Experiment 1*: 0.008–0.029 *Experiment 2*: 0.008–0.025
s-methoprene	50 g l^{-1}	ProLink™ Liquid Larvicide	Pacific Biologics	0.00367–0.0367
temephos	97.5%	Pestanal®, analytical standard	Fluka, Sigma-Aldrich	0.001 diagnostic/comparison dose

Table 3. Knockdown concentrations of bifenthrin (ppm) for *Aedes aegypti* at 1 and 24 h after exposure from lines infected with *w*MelPop or *w*Mel compared with uninfected (u) lines – confidence intervals and heterogeneity factor were generated by Probit analysis.

Line	*n*	Controls	Time (h)	KD30 ppm (95% CI)	KD50 ppm (95% CI)	KD90 ppm (95% CI)	Slope (SE)	Hetero-geneity
Comparison 1: *w*MelPop								
C2MB (u)	364	52	1	0.013 (0.008–0.018)	0.027 (0.019–0.035)	0.155 (0.105–0.281)	1.678 (±0.195)	1.30
AOC2	251	36	1	0.037 (0.026–0.048)	0.061 (0.047–0.083)	0.212 (0.142–0.419)	2.378 (±0.312)	1.32
BUC2	140	20	1	0.017 (0.010–0.024)	0.030 (0.021–0.041)	0.107 (0.071–0.234)	2.288 (±0.429)	0.81
C2MB (u)	364	52	24	0.014 (0.008–0.019)	0.027 (0.020–0.035)	0.143 (0.098–0.258)	1.765 (±0.208)	1.30
AOC2	251	36	24	0.029 (0.021–0.037)	0.053 (0.041–0.069)	0.232 (0.158–0.416)	1.994 (±0.248)	0.98
BUC2	140	20	24	0.015 (0.007–0.022)	0.026 (0.016–0.037)	0.091 (0.057–0.261)	2.329 (±0.459)	0.98
Comparison 2: *w*MelPop								
C67 (u)	179	26	1	0.018 (0.007–0.030)	0.033 (0.018–0.056)	0.141 (0.077–0.623)	2.038 (±0.323)	2.40
O1MB	142	19	1	0.024 (0.011–0.036)	0.040 (0.025–0.065)	0.147 (0.084–0.571)	2.274 (±0.411)	1.71
AOMB	183	25	1	0.041 (0.032–0.049)	0.054 (0.045–0.066)	0.106 (0.083–0.164)	4.384 (±0.775)	0.72
BOMB	180	25	1	*	*	*	*	*
C67 (u)	179	26	24	0.022 (0.013–0.031)	0.035 (0.024–0.046)	0.101 (0.072–0.198)	2.755 (±0.565)	0.99
O1MB	142	19	24	0.018 (0.003–0.037)	0.038 (0.013–0.078)	0.240 (0.108–1.876)	1.593 (±0.281)	2.27
AOMB	183	25	24	0.021 (0.015–0.027)	0.032 (0.025–0.041)	0.090 (0.066–0.153)	2.873 (±0.475)	0.86
BOMB	180	25	24	0.012 (0.004–0.018)	0.020 (0.010–0.029)	0.070 (0.044–0.208)	2.339 (±0.439)	1.78
Comparison 3: *w*MelPop								
C67 (u)	251	38	1	0.022 (0.006–0.048)	0.079 (0.035–0.155)	1.741 (0.700–9.553)	0.954 (±0.112)	2.34
O1MB	244	34	1	0.012 (0.002–0.029)	0.048 (0.017–0.098)	1.350 (0.511–9.706)	0.882 (±0.117)	2.31
U3MB	247	37	1	0.008 (0.001–0.022)	0.033 (0.009–0.075)	1.207 (0.427–11.711)	0.822 (±0.115)	2.46
AOMB	250	36	1	*	*	*	0.390 (±0.095)	2.39
BOMB	249	35	1	0.010 (0.001–0.026)	0.047 (0.015–0.100)	2.082 (0.704–20.653)	0.777 (±0.110)	2.11
C67 (u)	251	38	24	0.016 (0.012–0.019)	0.022 (0.018–0.026)	0.049 (0.040–0.068)	3.628 (±0.522)	1.00
O1MB	244	34	24	0.021 (0.014–0.027)	0.034 (0.026–0.044)	0.121 (0.086–0.212)	2.352 (±0.365)	0.73
U3MB	247	37	24	0.015 (0.011–0.019)	0.023 (0.018–0.028)	0.063 (0.049–0.094)	2.943 (±0.418)	0.76
AOMB	250	36	24	0.014 (0.010–0.018)	0.020 (0.016–0.025)	0.048 (0.038–0.071)	3.435 (±0.550)	1.02
BOMB	249	35	24	0.017 (0.013–0.020)	0.023 (0.019–0.028)	0.052 (0.042–0.073)	3.678 (±0.504)	0.84
Comparison 1: *w*Mel								
C89 (u)	178	25	1	0.029 (0.013–0.046)	0.047 (0.028–0.080)	0.145 (0.083–0.645)	2.605 (±0.408)	2.86
*w*Mel	139	20	1	0.025 (0.010–0.044)	0.053 (0.028–0.095)	0.309 (0.153–1.469)	1.665 (±0.268)	1.82
*w*C89	176	25	1	0.039 (0.031–0.047)	0.050 (0.042–0.059)	0.089 (0.072–0.128)	5.140 (±0.930)	0.71
C89 (u)	178	25	24	*	*	*	*	*
*w*Mel	139	20	24	0.014 (0.003–0.025)	0.026 (0.010–0.045)	0.125 (0.068–0.586)	1.893 (±0.418)	1.47
*w*C89	176	25	24	0.021 (0.014–0.027)	0.031 (0.024–0.040)	0.081 (0.059–0.144)	3.083 (±0.507)	1.10
Comparison 2: *w*Mel								
C89 (u)	245	35	1	0.004 (0.000–0.013)	0.019 (0.003–0.047)	0.963 (0.335–10.455)	0.750 (±0.116)	2.28
*w*Mel	250	36	1	0.002 (0.000–0.010)	0.019 (0.002–0.050)	2.895 (0.780–78.296)	0.585 (±0.106)	1.77
*w*C89	248	35	1	0.004 (0.000–0.013)	0.021 (0.004–0.052)	1.416 (0.474–16.323)	0.702 (±0.111)	2.00
C89 (u)	245	35	24	0.010 (0.005–0.014)	0.016 (0.010–0.021)	0.048 (0.035–0.081)	2.654 (±0.466)	1.14
*w*Mel	250	36	24	0.009 (0.005–0.012)	0.014 (0.009–0.018)	0.044 (0.033–0.069)	2.540 (±0.437)	0.93
*w*C89	248	35	24	0.006 (0.003–0.010)	0.011 (0.006–0.015)	0.037 (0.027–0.063)	2.371 (±0.441)	1.15

were dispensed into each jar using a 10-ml pipette. Five replicates of each concentration were made. Lids were placed on jars. Mortality was scored at 24 and 48 h. Temperature throughout the bioassay was variable due to a cabinet program malfunction and ranged from 22–25°C (no light). Data were subjected to probit analysis using PoloPlus Version 2 (© 2002–2011 LeOra Software). Lines tested were C67, C89 (field origin), O1MB, U3MB (*w*MelPop strain), AOMB, BOMB

Table 4. Bioassays of *Aedes aegypti* with VectoBac® WG *Aedes aegypti* 48 h 26°C (BC, backcrossed line: line backcrossed to field line) (Comparison 1)−confidence intervals and heterogeneity factor were generated by Probit analysis.

Date	Line	Status	*n*	Controls	LC_{30} ppm	Lower 95% CI	Upper 95% CI	LC_{50} ppm	Lower 95% CI	Upper 95% CI	LC_{90} ppm	Lower 95% CI	Upper 95% CI	Slope	SE	Heterogeneity
04/03/2010	C45	Uninfected	316	40	0.019	0.014	0.022	0.025	0.021	0.031	0.048	0.036	0.124	4.4	0.7	3.29
04/02/2010	*w*Mel	*w*Mel	350	40	0.018	0.015	0.020	0.022	0.020	0.024	0.036	0.032	0.045	6.3	0.8	2.02
04/03/2010	*w*Mel	*w*Mel	315	40	0.013	0.006	0.017	0.019	0.013	0.023	0.042	0.031	0.120	3.6	0.5	4.18
17/09/2010	*w*Mel	*w*Mel	175	25	0.009	0.004	0.012	0.012	0.007	0.015	0.024	0.019	0.035	4.2	0.8	2.09
04/03/2010	*w*C45	*w*MelBC	318	40	0.017	0.012	0.020	0.023	0.019	0.027	0.044	0.034	0.095	4.4	0.6	3.69
17/09/2010	O1MB	*w*MelPop	175	27	0.010	0.007	0.013	0.012	0.009	0.015	0.020	0.017	0.025	6.1	1.0	1.59
04/03/2010	U3MB	*w*MelPop	321	40	0.020	0.015	0.022	0.024	0.021	0.027	0.039	0.033	0.064	6.1	1.0	2.63
17/09/2010	AOMB	*w*MelPopBC	175	25	0.011	0.009	0.013	0.013	0.011	0.015	0.018	0.017	0.021	8.7	1.6	0.93
17/09/2010	BOC2	*w*MelPopBC	175	25	0.012	0.007	0.014	0.014	0.009	0.017	0.022	0.018	0.028	6.6	1.1	1.98

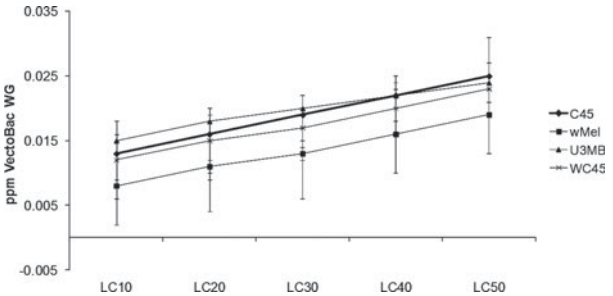

Fig. 1. Susceptibility of lines of *Aedes aegypti* to *Bacillus thuringiensis* subsp. *israelensis* (VectoBac® WG) (48 h, 26°C) $-LC_{10}$ to LC_{50} with 95% confidence intervals (Comparison 1). Confidence intervals were generated by Probit analysis using PoloPlus.

(*w*MelPop on field background), *w*Mel and *w*C89 (*w*Mel on field background).

s-methoprene (larvae)

In the first comparison of strains, reverse osmosis water (450 ml) was dispensed into 35 1-l rectangular plastic containers (Anchor Packaging, Rosebery, NSW, Australia) to which ¼ of a crushed TetraMin® tablet was added. Forty larvae of *Ae. aegypti* were placed into each container using a tea strainer. The s-methoprene solutions (50 ml) (table 1) were dispensed into each container (7 lines × 5 concentrations). Lines tested were C67 (field origin), O1MB (*w*MelPop line), AOMB (*w*MelPop on field background), BOMB (*w*MelPop on field background), C89 (field origin), *w*Mel, and *w*C89G1 (*w*Mel on field background). Forty larvae were tested per concentration for each line and for a RO water control. Temperature throughout the bioassay was 26.5 ± 1°C (no light).

In the second comparison, reverse osmosis water (189 ml) was dispensed into 250-ml glass jars (Cospak Pty Ltd). Ten larvae of *Ae. aegypti* (three days since hatching) were placed into each jar using a tea strainer; 1 ml of a food solution comprising 12 TetraMin® tablets crushed and suspended in 240 ml RO water was added to each jar. Solutions of a range of concentrations of s-methoprene were dispensed into each jar in 10-ml aliquots to make final concentrations of (0.0037–0.0370 ppm). Lines tested were C67 (field origin), O1MB (*w*MelPop line), U3MB (*w*MelPop line), AOMB (*w*MelPop on field background), BOMB (*w*MelPop on field background), C89 (field origin), *w*Mel, and *w*C89 (*w*Mel on field background). Fifty larvae were tested per concentration for each line and for a RO water control. The jars were sealed with plastic screw-top lids. Temperature throughout the bioassay was 26.5 ± 1°C (no light). Larvae were fed with Tetramin® crushed to a powder (approx. six tablets over 240 jars) at 72 h.

Temephos (larvae)

Reverse osmosis water (249 ml) was dispensed into 250-ml glass jars (Cospak Pty Ltd). Twenty larvae (three days after hatching) were placed in each jar using a tea strainer. A solution of temephos dissolved in absolute ethanol (1 ml) was dispensed into each jar. The control comprised 1 ml absolute

Table 5. Effect of temephos (0 & 0.001 ppm, 24 h, 26°C) on *Aedes aegypti* (*Wolbachia*-infected vs uninfected larvae).

Line	ppm	*n*	% mortality	% moribund	% alive not moribund
AOMB	0	101	0.0	0.0	100.0
BOMB	0	101	0.0	0.0	100.0
C67	0	99	0.0	0.0	100.0
C89	0	100	0.0	0.0	100.0
O1MB	0	100	0.0	0.0	100.0
U3MB	0	102	0.0	0.0	100.0
*w*C89	0	101	0.0	0.0	100.0
*w*Mel	0	98	0.0	1.0	99.0
AOMB	0.001	110	41.8	33.6	24.5
BOMB	0.001	100	38.0	28.0	34.0
C67	0.001	102	32.4	29.4	38.2
C89	0.001	103	31.1	35.9	33.0
O1MB	0.001	105	51.4	27.6	21.0
U3MB	0.001	101	61.4	19.8	18.8
*w*C89	0.001	97	45.4	26.8	27.8
*w*Mel	0.001	101	46.5	18.8	34.7

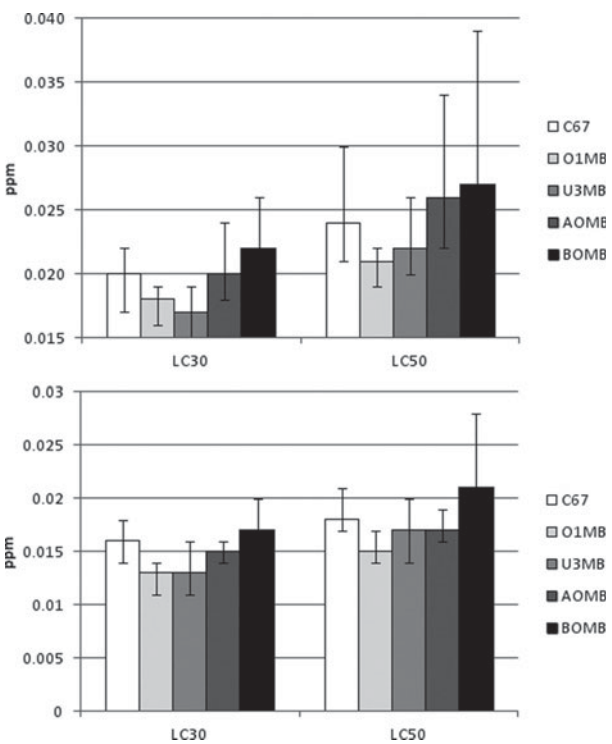

Fig. 2. LC_{30} and LC_{50} (with 95% confidence intervals) of VectoBac® WG for lines of *Aedes aegypti* infected with *w*MelPop (C67 is the uninfected line) at 24 h and 48 h. Confidence intervals were generated by Probit analysis using PoloPlus.

ethanol in 249 ml RO water. One hundred larvae from each line were tested at 0 ppm and 0.001 ppm temephos. Lines tested were C67 (field origin), O1MB (*w*MelPop line), AOMB (*w*MelPop on field background), BOMB (*w*MelPop on field background), C89 (field origin), *w*Mel, and *w*C89G1 (*w*Mel on field background). The jars were sealed with plastic screw-top lids. Mortality was scored at 24 h. Temperature throughout the bioassay was 26.5 ± 1°C (no light).

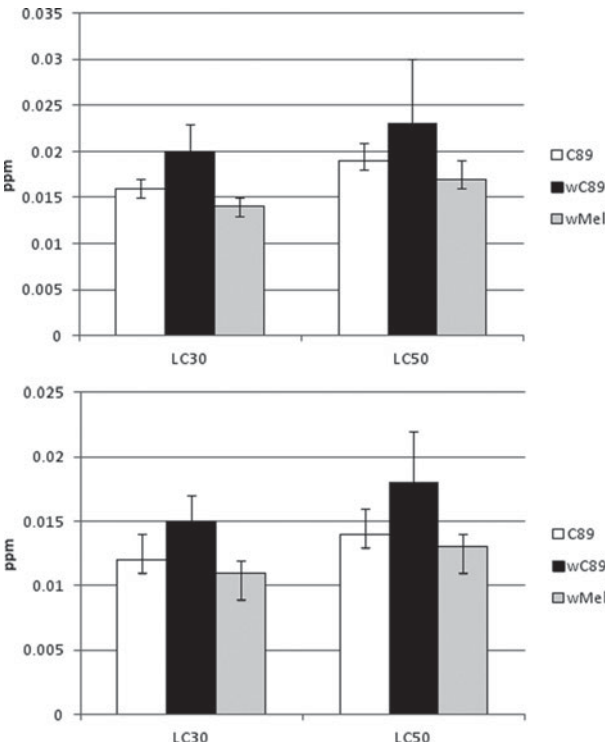

Fig. 3. LC_{30} and LC_{50} (with 95% confidence intervals) of VectoBac® WG for lines of *Aedes aegypti* infected with *w*Mel (C89 is the uninfected line) at 24 h and 48 h. Confidence intervals were generated by Probit analysis using PoloPlus.

Results

Bifenthrin (adults)

Some insects knocked down at 1 h had recovered by 24 h after exposure. All insects that were still knocked down at 24 h appeared moribund. KD_n is equal to the concentration required to knock down n% of the population (table 2).

In the first comparison with *w*MelPop, a low number of individuals from each population was able to recover after being knocked down (AOC2: 7.8%, BUC2: 4.9% and C2MB: 4.8%). BUC2 and C2MB had a very similar response to bifenthrin. AOC2 mosquitoes were slightly more tolerant to bifenthrin than were those from BUC2 and C2MB at KD_{30} and KD_{50} with non-overlapping 95% confidence intervals with other lines as determined by Probit analysis (table 3). In the second *w*MelPop comparison, at 24 h (table 3), for KD_{30} and KD_{50}, there were no differences between lines in response to bifenthrin (95% confidence intervals overlap). Finally, in the third comparison, O1MB, the laboratory *w*MelPop line, was more tolerant to bifenthrin than were mosquitoes from AOMB and BOMB, the outbred *w*MelPop lines, at 24 h at KD_{50} and KD_{90} (table 3).

For the first comparison with *w*Mel, there was no difference in response to bifenthrin (i.e. 95% confidence intervals overlap) of C89 (uninfected line), *w*Mel and *w*C89 at KD_{30}, KD_{50} and KD_{90}, with the exception of *w*C89 being more susceptible than C89 at KD_{90} (1 h) (table 3). For the second comparison, there was no difference in response to bifenthrin (i.e. 95% confidence intervals overlap) of C89 (uninfected

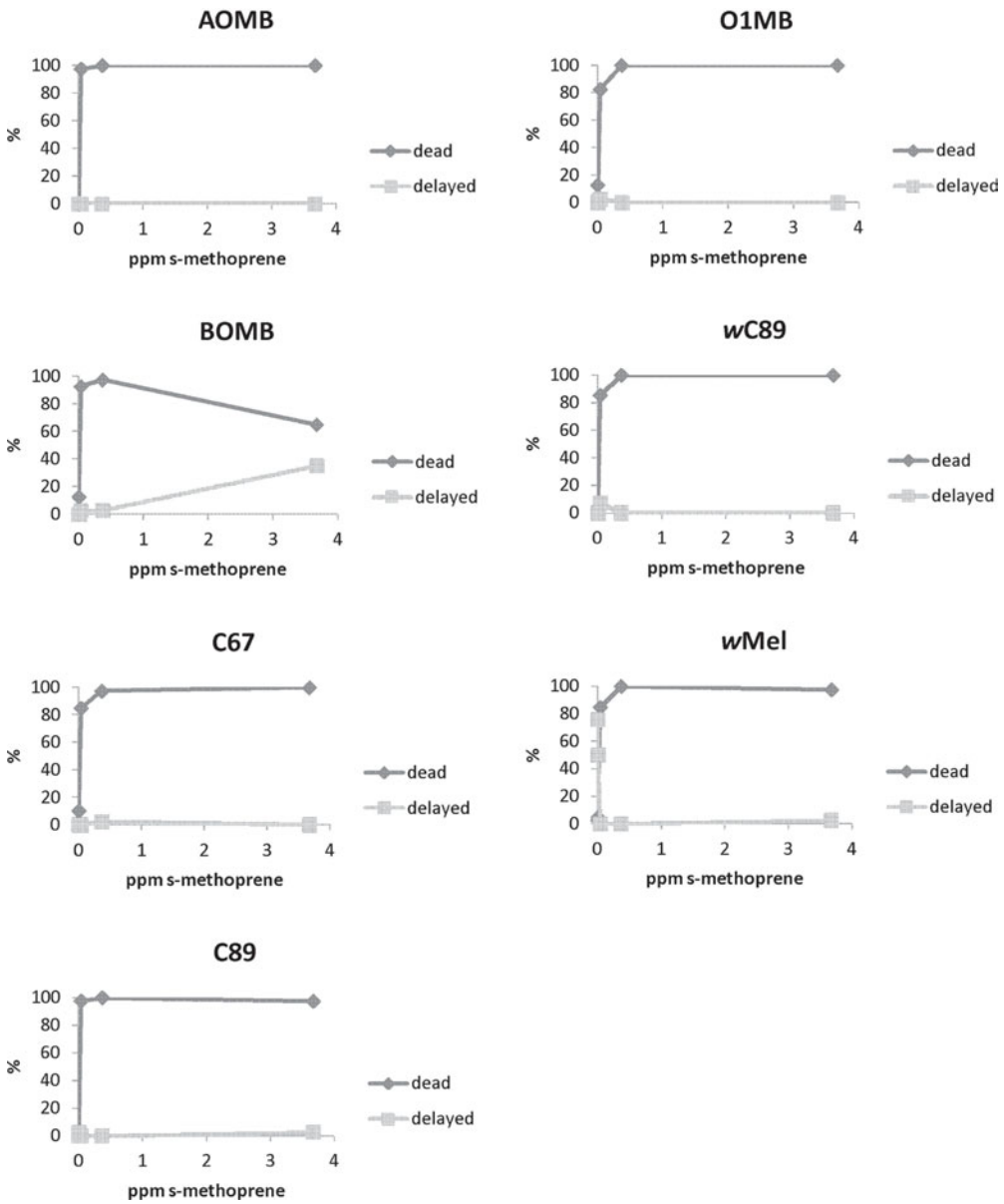

Fig. 4. Percentage mortality and delayed development of *Aedes aegypti* larvae exposed to s-methoprene for 13 days (Comparison 1).

line), *w*Mel and *w*C89 at KD_{30}, KD_{50} and KD_{90} at 1 h and 24 h (table 3). These data suggest no difference between infected and uninfected lines with respect to response to bifenthrin.

Bacillus thuringiensis *(larvae)*

In the first comparison, after 48 h exposure to VectoBac® WG, there was no difference between lines C45, *w*C45, *w*Mel and U3MB, for $LC_{10, 20, 30, 40,}$ and $_{50}$ (95% confidence intervals overlapped) (fig. 1). There was also no difference in response to VectoBac® WG between the AOMB and BOC2 lines (table 4). There was one incidence of the *w*Mel, O1MB, AOMB and BOC2 lines showing greater susceptibility to VectoBac® WG than the C45 line, though this was across bioassay dates (table 4) .

In the second comparison, there were no consistent differences between lines of *Ae. aegypti* infected with *Wolbachia* and uninfected lines with respect to toxicity of *B. thuringiensis* at 24 h and 48 h. There were some differences between infected lines; for example, the laboratory line infected with *w*MelPop, O1MB, appears to be more susceptible than BOMB, the *w*MelPop line backcrossed to the field line (24 h: LC_{30} and LC_{50}, and at 48 h: LC_{50} only) (fig. 2). The outbred line, *w*C89, shows higher LC_{30} and LC_{50} than the *w*Mel laboratory line (fig. 3).

s-methoprene (larvae)

For the first comparison, a graph of percentage mortality and delayed development of the larvae from each line after

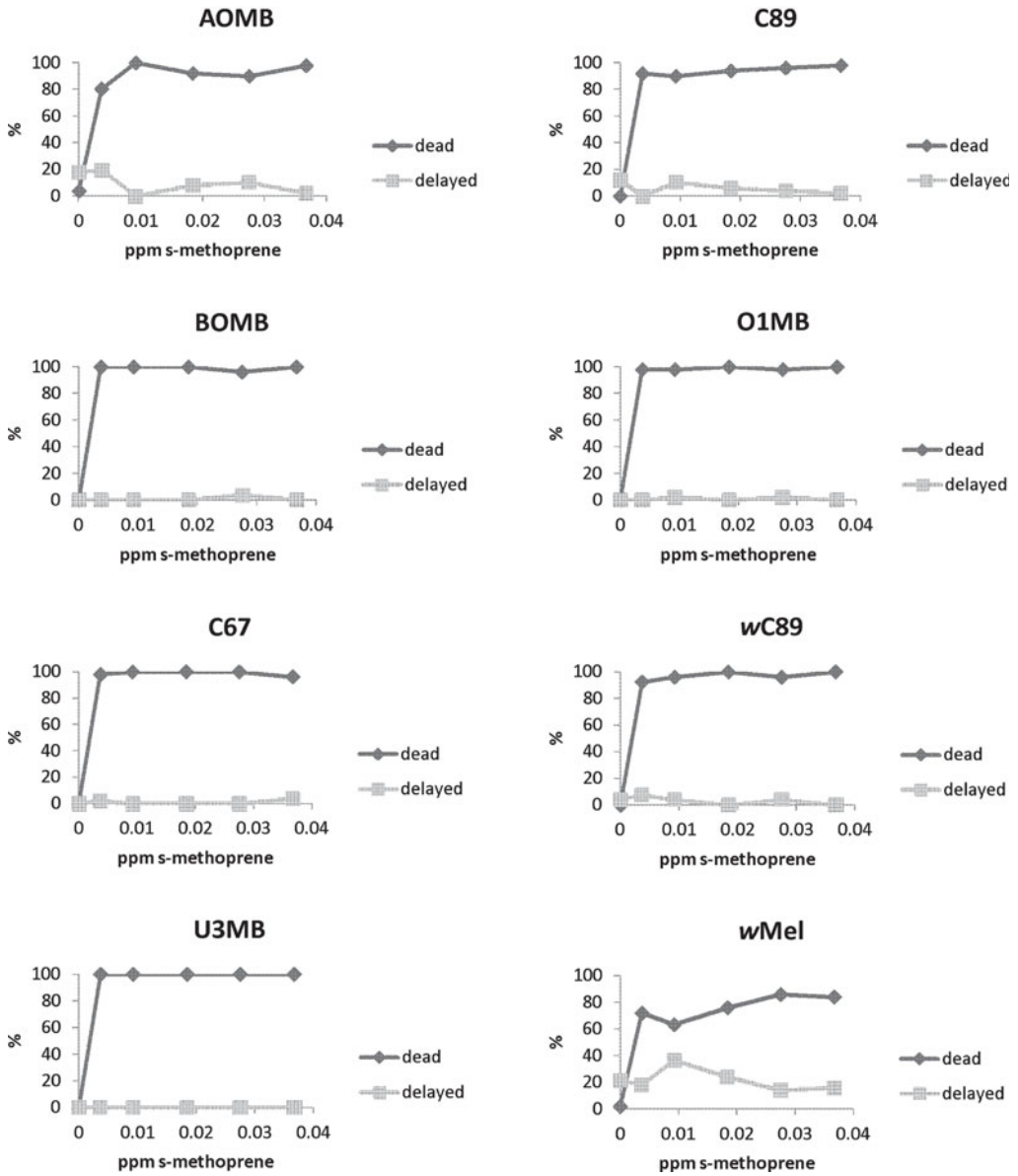

Fig. 5. Percentage mortality and delayed development of *Aedes aegypti* larvae exposed to s-methoprene for 13 days (Comparison 2).

13 days (fig. 4) shows that there was very little control mortality (controls: RO water only) and that there were levels of delayed development (i.e. larvae that did not pupate) in some of the lines that were unrelated to treatment with s-methoprene (C89, AOMB, *w*Mel, *w*C89). Overall, levels of mortality were high throughout the entire range of concentrations (0.000367 to 0.036700 ppm) for every line. A small number of adults eclosed successfully, but only from the lowest concentration and for the lines C89, O1MB and *w*Mel (fig. 4). There were some incidences of delayed development at each concentration of s-methoprene, but this was most pronounced in *w*Mel and cannot be attributed directly to s-methoprene because of the behaviour of this line in the control. With respect to final fate attributable to s-methoprene, there is no apparent distinction between *Wolbachia* or non-*Wolbachia* lines.

For the second comparison, at concentrations of 0.367 ppm and above, there was no successful emergence of adults (fig. 5). Very few adults emerged at 0.0367 ppm. Development of larvae from the *w*Mel line was delayed in the control. At the highest concentration, the BOMB line showed a high percentage of larvae which did not pupate. These data do not suggest that there is any difference between *Wolbachia*-infected and uninfected lines with respect to response to s-methoprene.

Temephos (larvae)

A dose-response was not sought for this insecticide as a pilot assay was not able to differentiate between a series of concentrations lower than 0.001 ppm. Instead, larvae from each line were either tested at 0 ppm or 0.001 ppm (table 4).

At these concentrations, there appear to be no differences in responses between *Wolbachia*-infected and uninfected lines of *Ae. aegypti* (Kruskal-Wallis Test $P = 0.378$ (mortality), $P = 0.742$ (moribund)) (table 5).

Discussion

Differences between lines were present for some insecticides, suggesting genetic variation among the lines in responses to chemicals. However, there were no obvious or consistent differences between *Wolbachia* infected and uninfected lines. Of the ten comparisons completed overall, infected lines performed better on average than uninfected lines in only three of these. Mortality rates were also similar to those expected, based on other studies. Thus, there was no evidence of highly insecticide-resistant lines in this study and no evidence for an effect of *Wolbachia* infection on insecticide susceptibility.

These results suggest that *Wolbachia* introductions will not adversely influence chemical control of *Ae. aegypti*. In North Queensland where *Wolbachia* invasions are currently being undertaken, chemical applications are used following detection of dengue cases based on applications of the chemicals tested here (http://www.health.qld.gov.au/dengue/managing_outbreaks/mosquito.asps). Methoprene, Bt and pyrethroids, as well as organophosphates, continue to be used in other parts of the world for *Aedes* control, despite the emergence of resistance problems and potentially negative environmental effects (Ranson *et al.*, 2010).

The small differences in pesticide response between lines are unlikely to be important from a control perspective. Chemical resistance in *Ae. aegypti* typically involves much larger differences among populations than detected here, involving shifts in LD_{50} of 5–10 fold or more (Rodriguez *et al.*, 2007). The small effects we found are unlikely to contribute to control failures; they may reflect an effect of laboratory culture because in some cases (e.g. figs 1, 3) the infected laboratory population had a lower level of resistance. It has previously been noted that long-term infected laboratory lines can have a lower fitness than infected outcrossed lines (Yeap *et al.*, 2011), perhaps as a consequence of inbreeding depression. It is not likely that minor differences between lines are caused by differences in other symbionts or microbial communities within the lines, given the crossing schemes undertaken to create them.

Wolbachia infection confers some resistance to insect pathogens of *Drosophila melanogaster* (particularly the entomopathogenic fungus, *Beauveria bassiana*) (Panteleev *et al.*, 2007) and is also known to provide protection against RNA viruses in this species (Teixeira *et al.*, 2008). *Wolbachia* also provide some protection against bacteria, although this depends on the population tested (Wong *et al.*, 2011). However, the present results indicate that this does not translate into increased resistance to toxins from the bacterial insect pathogen, *B. thuringiensis*.

In conclusion, *Wolbachia* do not influence resistance to chemicals used to treat mosquito infestations including larvicides. This means that successful invasion of natural populations by *Wolbachia* is unlikely to adversely affect the potential of authorities to use other control methods. *Wolbachia* invasions may well reduce the need for chemical options, given its ability to suppress transmission of dengue and also potentially other disease agents.

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