Limited impacts of truck-based ultra-low-volume applications of mosquito adulticides on mortality in honey bees (*Apis mellifera*)

**F.D. Rinkevich[1,2]†, J.W. Margotta[1], V. Pokhrel[1],
T.W. Walker[3], R.H. Vaeth[3], W.C. Hoffman[4], B.K. Fritz[4],
R.G. Danka[2], T.E. Rinderer[2], R.L. Aldridge[5],
K.J. Linthicum[5], J.A. Ottea[1] and K.B. Healy[1]***

[1]Department of Entomology, Life Sciences Annex, Agricultural Center, Louisiana State University, Baton Rouge, LA 70803, USA: [2]Honey Bee Breeding, Genetics, and Physiology Laboratory, USDA-ARS, 1157 Ben Hur Rd, Baton Rouge, LA 70820, USA: [3]East Baton Rouge Parish Mosquito Abatement and Rodent Control, 2829 Lt Gen Ben Davis Jr Ave, Baton Rouge, LA 70807, USA: [4]USDA-ARS, Aerial Application Technology Research Unit, 2881 F and B Rd, College Station, TX 77845, USA: [5]USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, FL 32608, USA

Abstract

Adulticides applied against mosquitoes can reduce vector populations during times of high arbovirus transmission. However, impacts of these insecticides on pollinators and other non-target organisms are of concern to mosquito control professionals, beekeepers and others. We evaluated mortality of *Culex quinquefasciatus* and *Apis mellifera* when caged insects were exposed to low and high label rates of four common adulticides (Aqua-Pursuit™ [permethrin], Duet® [prallethrin + sumithrin], Fyfanon® [malathion] and Scourge® [resmethrin]) at six distances up to 91.4 m from a truck-mounted ultra-low-volume sprayer. Honey bee mortality was both absolutely low (<10%) and low relative to mosquito mortality for most products, distances, and application rates. Exceptions were at the high rate of Fyfanon (honey bee mortality of 22–100% at distances ≤61 m) and the low rate of Scourge (mortality <10% for both insects). The greatest ratios of mosquito-to-honey bee mortality were found for the low rate of Fyfanon (30× greater) and the high rate of Duet (50× greater). Aqua-Pursuit and Fyfanon tended to increase mortality of both species at closer distances and at higher application rate; this was related to increased number and size of spray droplets. Wind speed and temperature had inconsistent effects on mortality of mosquitoes only. In this bioassay designed to have insects directly intercept insecticide droplets, mosquito adulticides applied at low rates and at >61 m had limited impacts on honey bee mortality while providing effective mosquito control.

---

*Author for correspondence
Phone: (225)578–7386
Fax: (225) 578–2257
E-mail: KHealy@agcenter.lsu.edu
†Current association: USDA-ARS, Honey Bee Breeding, Genetics, and Physiology Laboratory, 1157 Ben Hur Rd, Baton Rouge, LA 70820.

Introduction

While most mosquito control programs use an integrated approach, application of adulticides can help to reduce high nuisance levels and minimize the risk of vector-borne diseases to humans and animals. Realizing the potential impact of pesticides on pollinators, mosquito control personnel make a concerted effort to minimize adulticide treatments on managed honey bees (*Apis mellifera* L.) within their spray zones.

Commercially kept honey bee colonies contain an average of six pesticides (Mullin *et al.*, 2010), and synergistic interactions between these pesticides have been demonstrated (Johnson *et al.*, 2009; Johnson *et al.*, 2013; Rinkevich *et al.*, 2015). Of the 121 pesticides found in honey bee colonies, several insecticides (i.e. malathion, permethrin, prallethrin, and phenothrin) are commonly used as mosquito adulticides (Mullin *et al.*, 2010; Long & Krupke, 2016). The effects of some of these mosquito adulticides on honey bees have been evaluated. For example, several mosquito adulticides, including malathion and chlorpyrifos, were highly toxic when caged honey bees were directly exposed to non-thermal aerosol applications (Atkins *et al.*, 1981). One hour after malathion application, residues on foraged pollen was sufficient to kill bees (Pankiw & Jay, 1992). In addition, acute bee kills have been reported to occur following mosquito adulticiding operations with naled or malathion (Dixon & Fingler, 1984; Hester *et al.*, 2001; Zhong *et al.*, 2003).

Although many studies have focused on the toxicity of mosquito control adulticides to honey bees, evaluating honey bee exposure remains one of the crucial areas of study. A typical swath with a truck-based application of ultra-low volume (ULV) of mosquito adulticides is 91.4 m, but many factors (e.g. wind, droplet size, temperature) can affect how far the product can travel and how much can be deposited (Schleier *et al.*, 2012). For example, malathion tends to be lethal to honey bees at short distances (i.e. <30 m) from the application site, but with a rapid fall-off in mortality at increasing distance (Colburn & Langsford, 1970; Caron, 1979). Many mosquito control programs create buffer zones around bee hives, and applicators turn off their sprayers near hive locations. However, there is little information to guide programs on the size of the buffer necessary to protect the bees.

The goals of this study were to assess the impacts of mosquito control adulticiding practices on honey bee health and to improve current BMPs (best management practices) for mosquito control programs and apiculture. Our hypotheses were that honey bee mortality decreases as the distance from the treatment source increases, and insecticide deposition, droplet size and density correlates with bee mortality. Given that honey bees are considered to be highly susceptible to many insecticides (Anderson & Atkins, 1968; Iwasa *et al.*, 2004; Johnson *et al.*, 2006), with commonly kept Italian bees having the highest sensitivity (Danka *et al.*, 1986; Laurino *et al.*, 2013; Rinkevich *et al.*, 2015), it is important to evaluate the effects of these products utilizing real-world application rates and techniques in the field.
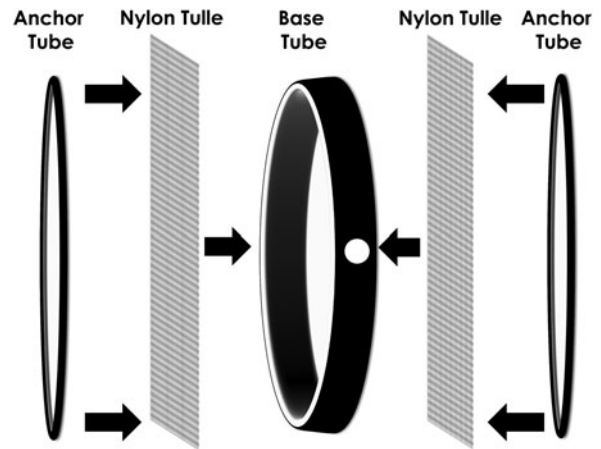

Fig. 1. Diagram of ring cages used to hold honey bees and mosquitoes. A 15.2 cm diameter mosquito ring cage (6″ Mosquito Ring Set, Pacific Paper Tube, Oakland, CA) was assembled with one base tube (14.9 cm inner diameter × 4.4 cm W × 0.2 cm thick with white liner inside) covered on both sides with nylon tulle (20.3 cm^2 of 0.1 cm mesh), then covered with two anchor tubes (15.2 cm inner diameter × 1.3 cm W × 0.2 cm thick). The base tube had a 2.5 cm hole punched in the side.

Materials and methods

Honey bees

Colonies of Italian honey bees (Wooten's Golden Queens, San Pedro, California, USA) were reared at the United States Department of Agriculture (USDA) Honey Bee Breeding, Genetics, and Physiology Laboratory in Baton Rouge, Louisiana, USA, without the use of miticides, antibiotics, or supplemental feed.

Forager bees were used in this study as they are the most likely members of a colony to encounter insecticides used in mosquito control applications. The colony entrance was blocked to cause an aggregation of returning foragers that could be scooped directly into a plastic container (38.1 cm H × 38.1 cm W × 60.0 cm L). A plastic funnel with a 1.9 cm opening was mounted in the corner at a 45° angle. The inside of the funnel was coated with a fine layer of petroleum jelly to easily add bees into a ring cage (fig. 1). The funnel of the bee box was placed through the hole of the ring cage. Approximately 30 bees were shaken in to the ring cage and the hole was plugged with 2 cotton balls soaked in 50% (w/v) sucrose solution. Caged bees were collected in mid-afternoon and held in a shaded location at ambient temperatures for 2–5 h before being used in spray trials in the evening.

Mosquitoes

Culex quinquefasciatus Say were reared in the insectary of the Mosquito and Fly Research Unit at the USDA-Agricultural Research Service (USDA-ARS) Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) in Gainesville, Florida USA. This strain of *C. quinquefasciatus* has been established in the insectary since 1995 from a Gainesville, Florida, USA, strain and was maintained using mass-rearing protocols (Gerberg *et al.*, 1994). The

Table 1. Insecticide product formulations and application parameters according to manufacturer's recommendations used in this study.

Product	Active ingredient (synergist)	Low rate (g ha^{-1})	High rate (g ha^{-1})
Aqua-Pursuit	Permethrin 20.6% (PBO 20.6%)	1.5 (1.5)	7.5 (7.5)
Duet	Prallethrin 1.0%,	0.28	0.82
	Sumithrin 5.0% (PBO 5.0%)	1.35 (1.35)	4.05 (4.05)
Fyfanon	Malathion 96.5%	35.0	67.5
Scourge	Resmethrin 4.1% (PBO 12.4%)	1.25 (3.75)	8.0 (24.0)

The formulated products containing the synergist piperonyl butoxide is abbreviated PBO. Labeled application rates were converted from lbs/acre to g/ha.

insecticide sensitivity of this strain was validated using CDC (Centers for Disease Control and Prevention) bottle bioassays (Brogdon & McAllister, 1998) at East Baton Rouge Parish Mosquito Abatement and Rodent Control (EBRPMARC) (Supplementary table 1).

The 1–2-day-old adult mosquitoes were shipped overnight to EBRPMARC, in Baton Rouge, Louisiana, USA. Ring cages (as above) were filled by aspirating approximately 20, 2–3-day-old female mosquitoes, and the hole was plugged with two cotton balls soaked in 10% (w/v) sucrose solution.

Field site

A 200 m × 100 m test plot was located at 30°30′56.4″N, 91° 09′12.6″W, approximately 3.2 km from the EBRPMARC and 1.0 km from the Baton Rouge Metropolitan Airport. The terrain was flat and covered by <0.25-m-high herbaceous vegetation. Three transects 91.4 m in length were oriented in the field so that wind traveled parallel to the transects, which were spaced 9.1 m apart. Each of the three transects had six stations at distances of 15.2, 30.5, 45.7, 61.0, 76.2, and 91.4 m from a perpendicular spray line. Each of the 18 stations contained a tower to hold insect-filled cages and a tower to hold rotating impingers. Cage towers were built of 1.9 cm PVC pipe with a 1.0 m cross bar to hold two cages at 1.5 m above the ground. Ring cages were attached to cage towers by spring-loaded hair clips. Cages were oriented perpendicular to the ground with the screened sides facing parallel to the spray line. Each cage tower at each station held one cage of mosquitoes and one cage of honey bees. Droplet collection towers housed rotating impingers (Leading Edge Associates Inc, Fletcher, North Carolina, USA) that held two Teflon-coated slides that were used to measure the number and size of droplets deposited.

Products tested

We evaluated the impacts of Aqua-Pursuit™ (permethrin 20.6%), Duet® (prallethrin 1.0%, sumithrin 5.0%), Fyfanon® (malathion 96.5%), and Scourge® (remethrin 4.1%) with each formulated product applied at the low rate and high rate indicated on the product labels (table 1). All materials were applied according to the manufacturer's label with a truck-mounted ADAPCO Guardian 190 ES ULV sprayer (ADAPCO, Sanford, Florida, USA) powered by a 4-cycle, 19 hp (674.98 cc) Kawasaki FH601D engine that drove a Dresser Roots blower. The vehicle speed was 24 kph and the ULV sprayer flow rate was 295.7 ml min^{-1}. Droplet size at the nozzle was characterized by analyzing >1000 droplets with a KLD Labs Inc. DC-IV 'hot wire' system before field application. The droplet diameter parameters (i.e. D_{v50} and D_{v90}) for all products were <30 and 30< × <50 microns, respectively.

Non-metallic (thick-walled plastic) containers were used as treatment insecticide reservoirs (Briggs & Stratton Smart-Fill, 2 + Gallon; The Plastic Group, Inc., Willowbrook, Illinois, USA). These 7570 ml (256 oz plus) containers were used for each insecticide at each concentration (low concentration and high concentration) to prevent contamination between insecticide treatments and reduce the time required in the flushing of containers and lines. The changing of reservoir containers and the flushing of spray system lines were conducted off-site where there would be no chance for the drifting of the spray on to the study site as the result of the flushing operation. Low concentration treatments were applied before the high concentration treatments to reduce system flushing time.

When changing insecticide concentrations and insecticide treatments, the insecticide reservoir containers were disconnected from the spray system lines and the system was 'sprayed out' to remove residual insecticide within the lines. Before flushing of the spray system lines, the insecticide supply line, in-line filter reservoir was emptied between the different insecticide treatments. The system spray lines were flushed with isopropyl alcohol between the different insecticide treatments. The system was 'sprayed-out' after flushing. Spray system lines were not flushed between low and high concentration treatments of the same insecticide. When the new concentration or new insecticide treatment container was connected to the system the sprayer was allowed to spray for approximately 1 min to ensure the spray system was primed with the insecticide at the desired concentration.

All applications for each product and application rate were repeated at least three times between 6 and 8 p.m. with wind speed ranging between 0 and 9.3 km h^{-1} and temperature ranging between 22.8 and 29.0°C. Ambient weather conditions at 1 and 10 m above the ground were recorded by a portable Kestrel Weather station with a PC interface (Kestrel Meters, Birmingham, Michigan, USA). Duet®, Fyfanon®, and Scourge® trials were conducted during the week of 20 October 2014. Aqua-Pursuit™, Fyfanon®, and Scourge® trials were conducted during the week of 1 June 2015. The number of replicates performed for each species for each product at each application rate is shown on the corresponding figures.

Field procedures

As a control, four cages each of mosquitoes and honey bees along with four pairs of Teflon-coated slides were hung from towers adjacent (i.e. <10 m) to the transects. Control insects and slides were placed for 10 min before each application of insecticides. Honey bee and mosquito cages were collected in separate plastic garbage bags, and slides were collected in disposable slide boxes. Insect cages and slides were collected approximately 15 min after insecticide application. Insects

Table 2. Droplet, deposition, and environmental parameters for each product applied at low and high label rates.

Product	Rate	Deposition (nl cm^{-2})	D_{v50} (um)	D_{v90} (um)	Drops (n)	Wind (km h^{-1})	Temperature (°C)
Aqua-Pursuit	Low	0.9 (0.8–1.0)	31.5 (30.3–32.6)	47.9 (46.5–49.2)	34.9 (29.7–40.0)	1.9 (1.6–2.1)	25.0 (24.8–25.2)
	High	0.6 (0.5–0.7)	29.3 (27.8–30.8)	41.3 (39.2–43.4)	25.8 (23.7–28.0)	0	24.3 (24.0–24.6)
Duet	Low	0.4 (0.1–0.7)	24.9 (22.9–26.8)	38.7 (37.4–40.1)	32.8 (24.3–41.3)	5.6 (0)	24.4 (0)
	High	1.9 (1.0–2.8)	21.6 (20.3–23.0)	33.7 (31.0–36.4)	19.5 (15.2–23.8)	9.3 (0)	23.9 (0)
Fyfanon	Low	1.7 (0.7–2.6)	23.1 (22.0–24.1)	36.3 (33.8–38.9)	17.1 (14.7–19.6)	9.3 (0)	23.9 (0)
	High	1.1 (0.7–1.6)	30.7 (24.8–32.9)	48.5 (44.6–52.4)	34.3 (22.1–46.6)	5.6 (0)	24.0 (0)
Scourge	Low	0.3 (0.2–0.3)	26.8 (26.4–27.2)	40.3 (38.9–41.7)	18.0 (16.2–19.8)	1.8 (1.4–2.2)	28.1 (27.8–28.3)
	High	0.4 (0.4–0.5)	27.4 (26.9–27.9)	41.0 (39.6–42.3)	33.4 (31.8–35.0)	3.9 (3.5–4.3)	26.4 (26.4–26.5)

Values shown are the average (95% confidence interval).

were immediately transported back to EBRPMARC and transferred into clean holding containers within approximately 30 min after application. Clean holding containers consisted of a 475 ml wax paper cup (Inno-Pak, Delaware, OH) with a 2.5 cm hole punched in the wall and the top covered with nylon tulle secured by two rubber bands. Mosquitoes were transferred by mouth aspiration. Honey bees were transferred by funneling the bees from the cages into a clean holding container. The holes in the holding cages were plugged with two cotton balls soaked in 10 and 50% sucrose solution for mosquitoes and honey bees, respectively. The total number of insects in each holding cage was recorded. Mosquitoes were held at 22 ±3°C and 50±5% RH with seasonal daylight regime at in the research building EBRPMARC, while honey bees were held in an incubator in continuous darkness at 33 ± 1°C and 70 ± 5% RH. Insect mortality was recorded 24 h after treatment. Replicates where control mortality exceeded 10% were excluded from analysis.

Droplet deposition collection and analyses

Each of the 18 sampling stations included a tower holding two rotary impingers (spinners, Leading Edge Associates, Waynesville, North Carolina, USA). New slides were placed in each sampler before each spray pass. One of the spinners held two uncoated 25 mm × 75 mm glass microscope slides set 16 cm apart (outside edge to outside edge), with the slides rotated at a velocity of 5.6 m s^{-1} to capture airborne spray concentrations moving through the spray grid. The second spinner also held two 25 mm × 75 mm glass slides, but one slide was coated with Teflon tape and used in droplet sizing assessments. The uncoated slide remained throughout the trial to provide a counter balance to the coated slide. Exposed slides were collected individually into labeled, zip-top bags after each spray pass and after allowing sufficient time for the spray to travel through the grid. Zip-top bags containing exposed slides were stored in insulated containers for transport to the processing location.

Each spray mixture used included a fluorescent dye (Tinopal®, BASF, Research Triangle Park, North Carolina, USA) at a concentration of 5 g l^{-1} to allow for deposition analysis by fluorometry. Exposed slides were washed by adding 20 ml of hexane to each bagged sample and agitated for approximately 15 seconds. The 5 ml of the wash solution was decanted in borosilicate vials, which was processed for fluorescence using a spectrofluorophotometer (Model RF5000U, Shimadzu, Kyoto, Japan) with an excitation wavelength of 372 nm and an emission wavelength of 427 nm. The minimum detection level for Tinopal® is 0.00007 mg cm^{-2}. The

measured fluorescence values were converted to volume of spray solution using pre-mixed standard solutions of known dye concentration (Fritz et al., 2011). The resulting deposition data were in the form of total volume of spray solution per area sampled per slide surface.

Teflon-coated slides were analyzed within an hour after exposure using DropVision™ (Leading Edge Associates, LLC, Waynesville, North Carolina) by processing five rows of 12 images (Faraji et al., 2016). Low spray concentrations through the sampling grid resulted in a minimal numbers of droplets collected in individual slides (typically 50 or less), which required searching in a non-randomized pattern across the slides. Measurements were only made when droplets were found, which heavily biased coverage (drops per area) data. However, the droplet size results (i.e. D_{v50}, and D_{v90}) were representative of the spray moving through the sampling grid.

Statistics

Percentage mortality was calculated using Abbott's correction for control mortality (Abbott, 1925) that was arc-sine transformed. Honey bees and mosquito mortality at each distance was compared by ANOVA. Interactions between mortality and different factors (i.e. droplet size, concentration, wind, temperature, distance, and rate) were analyzed by General Linear Model. JMP® v12.1 statistical software (SAS, Cary NC) was used for all analyses.

Results

Deposition rate, droplet size (i.e. D_{v50}, D_{v90}), droplet number, wind speed, and temperature parameters for each field trial are shown in table 2. Average control mortality during the Aqua-Pursuit experiments was 0.0 and 3.3%, respectively, for honey bees and mosquitoes at the low rate, while control mortality was 8.3 and 3.3%, respectively, for honey bees and mosquitoes at the high rate. Honey bee mortality was significantly lower than mosquito mortality for Aqua-Pursuit at the low (df = 34, F = 25.6, P < 0.0001; fig. 2) and high rates of application (df = 29, F = 64.9, P < 0.0001; fig. 3) when mortality was pooled across all distances. Although mortality did not significantly differ between honey bees and mosquitoes at >61.0 m from the spray at the low rate, honey bee mortality was significantly lower than mosquito mortality as far as 76.2 m at the high application rate of Aqua-Pursuit (table 3). Honey bee mortality ranged from 17 to 42%, depending on distance, when exposed to high application rate of Aqua-Pursuit.

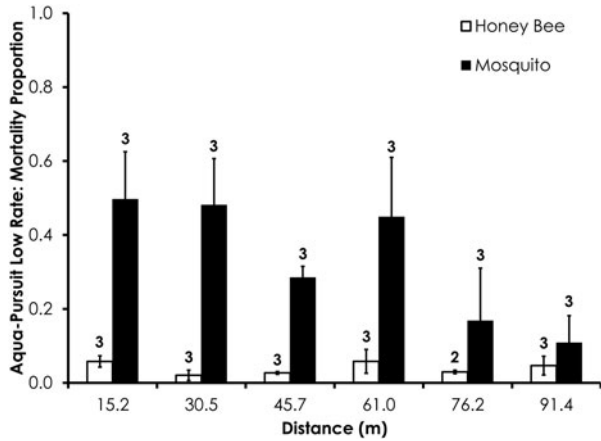Two replicates were removed from the Duet experiments due to high-control mortality (over 20%). After removal of

Fig. 2. Honey bee and mosquito mortality at distances from Aqua-Pursuit treatment at the low application rate. Control mortality for honey bees and mosquitoes was 0.0 and 3.3%, respectively. Data are shown as average mortality (±SEM). Numbers over mortality bars represent the number of replicates performed in triplicate.
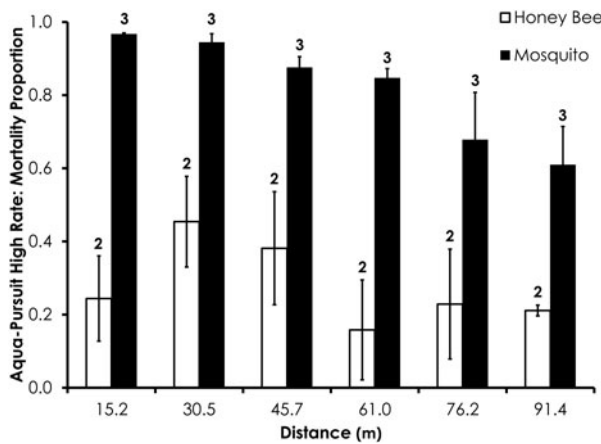

Fig. 3. Honey bee and mosquito mortality at distances from Aqua-Pursuit treatment at the high application rate. Control mortality for honey bees and mosquitoes was 8.3 and 3.3%, respectively. Data are shown as average mortality (±SEM). Numbers over mortality bars represent the number of replicates performed in triplicate.

those replicates, average control mortality during the Duet experiments was 0.0% for mosquitoes and honey bees at the low rate and 1.3 and 2.4% control mortality for honey bees and mosquitoes, respectively, the high Duet application rate. Overall, honey bee mortality was significantly lower than mosquito mortality for Duet for both low (df = 29, F = 20.4, P = 0.0001; fig. 4) and high rates of application (df = 47, F = 9.7, P = 0.0032; fig. 5) when mortality was pooled across all distances. Mortality did not significantly differ between the two species at >15.2 m from the spray at low and high application rates (table 3).

Two replicates were removed from the Fyfanon experiments due to high control mortality (over 20%). After removal, the average control mortality during the Fyfanon experiments

Table 3. Mortality differences between honey bee and mosquito for four commonly used mosquito adulticides at increasing distances from application site.

| Distance (m)	Aqua-Pursuit		Duet		Fyfanon		Scourge	
	Low	High	Low	High	Low	High	Low	High
15.2	df = 5, F = 11.5, P = 0.027	df = 4, F = 45.9, P = 0.007	df = 7, F = 8.9, P = 0.025	df = 4, F = 35.4, P = 0.009	df = 7, F = 3.4, P = 0.116	df = 7, F = 7.8, P = 0.031	df = 8, F = 0.4, P = 0.55	df = 9, F = 2.6, P = 0.144
30.5	df = 5, F = 13.2, P = 0.022	df = 4, F = 17.4, P = 0.025	df = 7, F = 2.3, P = 0.182	df = 4, F = 5.1, P = 0.110	df = 7, F = 0.7, P = 0.448	df = 7, F = 16.3, P = 0.007	df = 8, F = 3.3, P = 0.112	df = 9, F = 1.5, P = 0.248
45.7	df = 5, F = 71.4, P = 0.001	df = 4, F = 11.3, P = 0.043	df = 7, F = 2.9, P = 0.140	df = 4, F = 0.5, P = 0.519	df = 7, F = 0.7, P = 0.447	df = 7, F = 14.0, P = 0.009	df = 8, F = 4.7, P = 0.068	df = 9, F = 2.2, P = 0.178
61.0	df = 5, F = 5.6, P = 0.046	df = 4, F = 28.5, P = 0.013	df = 7, F = 2.1, P = 0.199	df = 4, F = 3.9, P = 0.141	df = 7, F = 0.6, P = 0.472	df = 7, F = 70.8, P < 0.001	df = 8, F = 1.1, =0.326	df = 9, F = 1.3, P = 0.288
76.2	df = 4, F = 0.6, P = 0.506	df = 4, F = 4.3, P = 0.129	df = 7, F = 0.1, P = 0.717	df = 4, F = 2.5, P = 0.214	df = 7, F = 1.4, P = 0.281	df = 7, F = 69.2, P < 0.001	df = 8, F = 2.0, P = 0.199	df = 9, F = 2.2, P = 0.17
91.4	df = 5, F = 0.6, P = 0.472	df = 4, F = 8.5, P = 0.042	df = 7, F = 0.1, P = 0.796	df = 4, F = 1.6, P = 0.297	df = 7, F = 0.8, P = 0.403	df = 7, F = 29.6, P = 0.0016	df = 8, F = 3.5, P = 0.101	df = 9, F = 1.8, P = 0.216
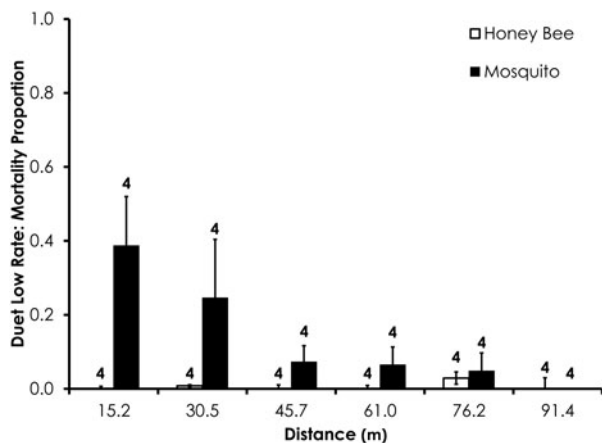
Fig. 4. Honey bee and mosquito mortality at distances from Duet treatment at the low application rate. Control mortality for both honey bees and mosquitoes was 0.0%. Data are shown as average mortality (±SEM). Numbers over mortality bars represent the number of replicates performed in triplicate.
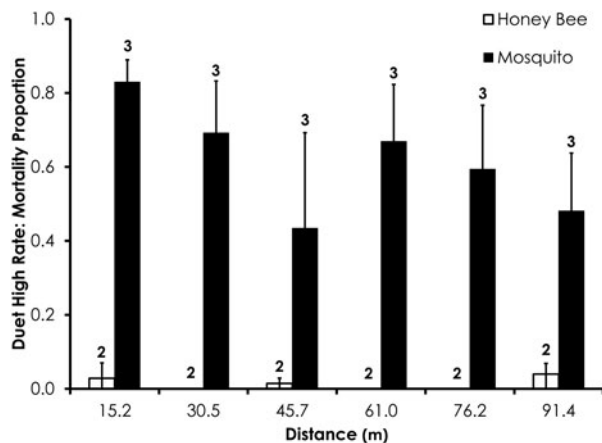

Fig. 5. Honey bee and mosquito mortality at distances from Duet treatment at the high application rate. Control mortality for honey bees and mosquitoes was 1.3 and 2.4%, respectively. Data are shown as average mortality (±SEM). Numbers over mortality bars represent the number of replicates performed in triplicate.

was 4.4 and 3.6% for honey bees and mosquitoes, respectively, at the low rate and 4.0 and 8.4% for honey bees and mosquitoes, respectively, at the high rate. Overall, honey bee mortality was significantly lower than mosquito mortality for Fyfanon for the low application rate (df = 47, $F = 7.96$, $P < 0.007$; fig. 6) when mortality was pooled across all distances, although there were no significant differences at any single distance (table 3). At the high Fyfanon application rate, honey bee mortality was significantly lower than mosquito mortality when comparing all samples pooled at all distances (df = 47, $F = 121.5$, $P < 0.0001$; fig. 7, table 3). Honey bee mortality was >80% at distances as far as 45.7 m when exposed to high-rate application of Fyfanon.

Average control mortality during the Scourge experiments was 1.9 and 4.5% for honey bees and mosquitoes at the low application rate. Control mortality was 6.9 and 0.0% for honey bees and mosquitoes, respectively, at the high Scourge application rates. Overall, honey bee mortality was significantly higher than mosquito mortality for Scourge at the low application rate (df = 53, $F = 13.4$, $P = 0.0006$; fig. 8, table 3) when mortality was pooled across all distances, although the average honey bee mortality was <13% for each distance. Honey bee mortality was significantly lower than mosquito mortality at the high rates of Scourge application distance (df = 59, $F = 12.9$, $P = 0.0007$; fig. 9, table 3) when mortality was pooled across all distances.

Factors that significantly influenced honey bee mortality varied by product (table 4). Aqua-Pursuit mortality was significantly increased by higher application rate, D_{v50}, and D_{v90}. An increase in D_{v50} was the only factor that significantly increased Duet mortality. Fyfanon mortality was significantly reduced by distance, but increased with high application rate, higher number of drops, and larger D_{v90}. No factor in our model was significant to determine mortality due to Scourge.

Mosquito mortality was significantly affected by more factors than honey bee mortality (table 5). Mortality due to Aqua-Pursuit was significantly reduced by farther distance, but increased at higher deposition, high application rate, and higher wind speed. No factor in our model was significant to determine mortality due to Duet. Fyfanon-induced mosquito
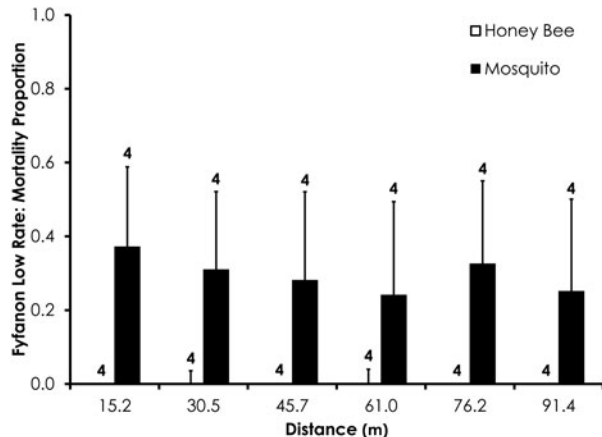

Fig. 6. Honey bee and mosquito mortality at distances from Fyfanon treatment at the low application rate. Control mortality for honey bees and mosquitoes was 4.4 and 3.6%, respectively. Data are shown as average mortality (±SEM). Numbers over mortality bars represent the number of replicates performed in triplicate.

mortality was significantly reduced by farther distance, but increased at higher deposition, and higher application rate. Higher deposition, application rate, wind speed, and temperature were significant factors that increased mosquito mortality due to Scourge.

Discussion

Our results showing lower honey bee mortality compared with mosquito mortality, especially at low application rates and far distances, agrees with other reports on the comparative toxicity of mosquito adulticides to honey bees and mosquitoes as well as the limited impacts of ULV applications. In controlled laboratory experiments, mosquitoes (*C. quinquefasciatus*) and honey bees (*A. mellifera*) had similar weight-
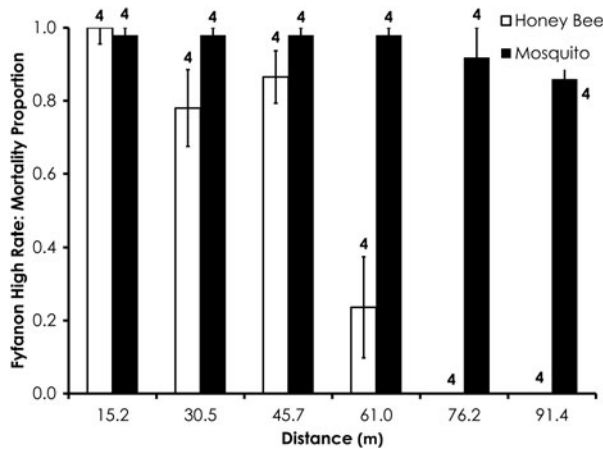
Fig. 7. Honey bee and mosquito mortality at distances from Fyfanon treatment at the high application rate. Control mortality for honey bees and mosquitoes was 4.0 and 8.4%, respectively. Data are shown as average mortality (±SEM). Numbers over mortality bars represent the number of replicates performed in triplicate.
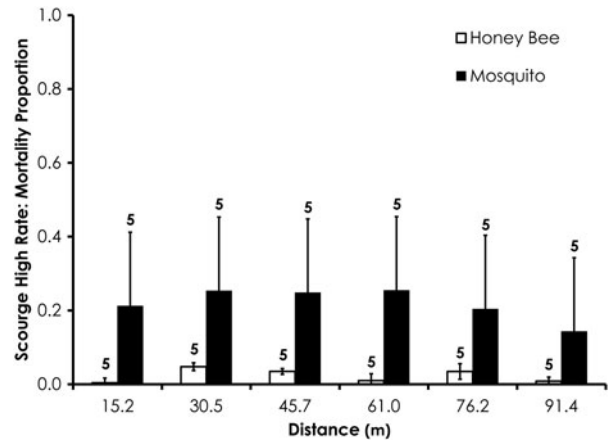

Fig. 9. Honey bee and mosquito mortality at distances from Scourge treatment at the high application rate. Control mortality for honey bees and mosquitoes was 6.9 and 0.0%, respectively. Data are shown as average mortality (±SEM). Numbers over mortality bars represent the number of replicates performed in triplicate.
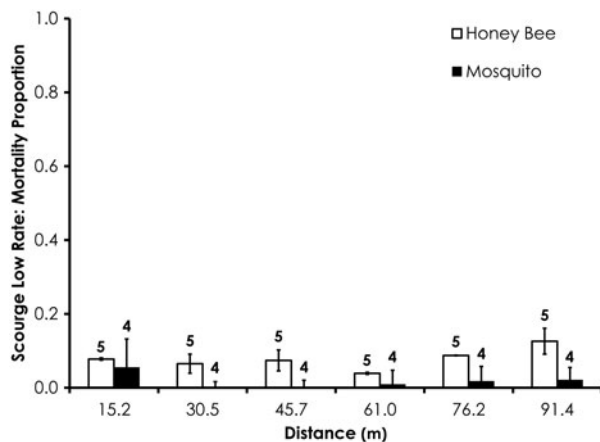

Fig. 8. Honey bee and mosquito mortality at distances from Scourge treatment at the low application rate. Control mortality for honey bees and mosquitoes was 1.9 and 4.5%, respectively. Data are shown as average mortality (±SEM). Numbers over mortality bars represent the number of replicates performed in triplicate.

standardized LD_{50} values for permethrin (Hardstone & Scott, 2010). Under field conditions in our experiments, however, mosquito mortality was significantly higher than honey bee mortality for the permethrin containing product, Aqua-Pursuit. This suggests that environmental factors such as distance, deposition, and wind were significant factors in determining mortality. Furthermore, Aqua-Pursuit is formulated with emulsifiers and adjuvants, as well as the synergist piperonyl butoxide (PBO). Formulated products tend to be significantly more toxic than technical materials (Seccacini *et al.*, 2008). We did observe significant bee mortality ranging from 17 to 42% with high doses of Aqua-Pursuit and more than 80% mortality as far as 45.7 m from the high-dose Fyfanon spray. Obviously care must be taken to avoid direct

spray contact with these two pesticides, especially at high application rates.

Large differences in live body weight likely accounts for much of the difference between honey bee and mosquito mortality. Honey bees weigh approximately 35 times more than mosquitoes by weight (82–93 mg for honey bee (Rinkevich *et al.*, 2015) and 2.5 mg for mosquitoes (AMCA mosquito.org/faq)). Weight is an important factor to consider when determining non-target impacts because heavier insects exhibit decreased sensitivity to ULV-applied insecticides (Schleier & Peterson, 2010; Chaskopoulou *et al.*, 2014). Therefore, caution should be exercised when extrapolating results from highly controlled laboratory studies with technical materials to variable field conditions with formulated products across species.

Distance from application site significantly impacted mosquito and honey bee mortality in this project. The effect of distance from the spray source on honey bee mortality has been previously reported (Hester *et al.*, 2001). In that study, overall honey bee mortality was very low, but it declined from 7.6 to 91.4 m from the application line of malathion, much like in our experiments with Fyfanon at the high application rate. In addition to low mortality in that study, there was no impact of malathion application on adult bee population or colony weight. These low impacts (especially at far distances) on honey bees occurred alongside very high mosquito mortality at the same distances. Distance is an important factor affecting mortality as deposition declines with distance. Distance and deposition were important factors for mosquito mortality due to Fyfanon, but only distance was a significant factor for honey bee mortality. Therefore, it appears that the decreased deposition that occurs with increasing distance from the spray line is not sufficient to affect honey bee mortality. Aerial application of Aqua-K-Othrine (2% deltamethrin) and Pesguard S102 (10% d-phenothrin) showed no significant mortality relative to the controls, and exposed colonies had the same number of frames of adult bees, brood population, and colony weight as the control (Chaskopoulou *et al.*, 2014). While aerial application of naled resulted in significantly higher numbers of dead honey bees than the control treatment,

Table 4. Significant factors that affect honey bee mortality across different products; interactions between these factors and mortality were analyzed by General Linear Regression.

Product	Distance	Deposition	Rate	Drops	D_{v50}	D_{v90}	Wind	Temperature
Aqua-Pursuit (106)	$F = 2.37, P = 0.12$	$F = 0.12, P = 0.72$	$F = 41.7, P < 0.001$	$F = 0.13, P = 0.71$	$F = 4.13, P = 0.04$	$F = 7.87, P = 0.005$	$F = 0.05, P = 0.82$	$F = 0.66, P = 0.42$
Duet (48)	$F = 0.41, P = 0.52$	$F = 0.47, P = 0.48$	$F = 2.58, P = 0.10$	$F = 1.32, P = 0.25$	$F = 3.87, P = 0.05$	$F = 4.16, P = 0.41$	NA	NA
Fyfanon (36)	$F = 15.1, P < 0.001$	$F = 0.02, P = 0.89$	$F = 9.31, P = 0.002$	$F = 21.4, P < 0.001$	$F = 0.79, P = 0.37$	$F = 4.83, P = 0.03$	NA	NA
Scourge (72)	$F = 0.48, P = 0.48$	$F = 2.36, P = 0.12$	$F = 3.58, P = 0.06$	$F = 0.60, P = 0.43$	$F = 0.46, P = 0.49$	$F = 1.25, P = 0.26$	$F = 1.03, P = 0.30$	$F = 0.53, P = 0.46$

The number under the product indicates how many data points were used in the analysis. The NA in Wind and Temperature columns are because there were no differences in wind and temperature parameters, so those data were not able to be used in the analysis.

Table 5. Significant factors that affect mosquito mortality across different products; interactions between these factors and mortality were analyzed by General Linear Regression.

Product	Distance	Deposition	Rate	Drops	D_{v50}	D_{v90}	Wind	Temperature
Aqua-Pursuit (108)	$F = 7.52, P = 0.006$	$F = 4.80, P = 0.03$	$F = 70.5, P < 0.001$	$F = 0.63, P = 0.42$	$F = 0.34, P = 0.55$	$F = 0.32, P = 0.57$	$F = 20.4, P < 0.001$	$F = 1.74, P = 0.18$
Duet (27)	$F = 1.17, P = 0.27$	$F = 0.88, P = 0.35$	$F = 0.94, P = 0.33$	$F = 3.61, P = 0.06$	$F = 0.90, P = 0.34$	$F = 0.01, P = 0.90$	NA	NA
Fyfanon (36)	$F = 3.93, P = 0.05$	$F = 17.1, P < 0.001$	$F = 46.0, P < 0.001$	$F = 1.74, P = 0.19$	$F = 2.40, P = 0.12$	$F = 0.98, P = 0.32$	NA	NA
Scourge (72)	$F = 0.51, P = 0.47$	$F = 5.60, P = 0.02$	$F = 6.93, P < 0.001$	$F = 2.51, P = 0.11$	$F = 0.02, P = 0.87$	$F = 0.69, P = 0.40$	$F = 29.1, P < 0.001$	$F = 17.7, P < 0.001$

The number under the product indicates how many data points were used in the analysis. The NA in Wind and Temperature columns are because there were no differences in wind and temperature parameters, so those data were not able to be used in the analysis.

exposed colonies produced as much honey as the control colonies (Zhong *et al.*, 2003). A small number of dead bees in proportion to colony size is likely to have little impact on an otherwise healthy colony as the number of workers that die from insecticide exposure would have to be more than twice the rate of those which die naturally for more than 35 days (Henry *et al.*, 2012). A prolonged exposure period is unlikely with these products as most of them rapidly degrade over a few hours, and they are not applied on a daily basis.

Increases in the D_{v50} and D_{v90} significantly increased honey bee mortality for Aqua-Pursuit, Duet, and Fyfanon, but did not significantly affect mosquito mortality. This demonstrates that larger droplets can result in higher honey bee mortality without any increase in effectiveness in mosquito control. Ensuring that application parameters are consistent with label recommendations may reduce non-target impacts while maintaining efficacious mosquito control.

The dramatic increase in honey bee mortality at close distances with the high rate of Fyfanon is consistent with the high slope of the bioassay dose response curve for malathion (Rinkevich *et al.*, 2015). This shows that a high level of accuracy must be exercised when using malathion containing products for ULV application to avoid the risks associated with high application rates.

It should be noted that these experiments were conducted in a fairly uniform field without obstructions of vegetation or structures, and with insects held in cages. It is likely that the application of these products in a realistic environment that may include buildings, trees, and honey bees in hives will undoubtedly affect the uniformity of product application, hazard to bees, and the efficacy against mosquitoes (Peterson *et al.*, 2016). Fortunately, because mosquito control occurs in the evening when bees are typically protected by the confines of the hive, and because of the rapid photodegradation of most mosquito control materials, the impacts of mosquito control operations on honey bee colonies is likely to be minimal when mosquito adulticides are properly applied. These compounding factors create a strong emphasis for future studies to evaluate actual exposure in real-world scenarios.

Supplementary Material

The supplementary material for this article can be found at https://doi.org/10.1017/S0007485317000347

Acknowledgements

References

Abbott, W.S. (1925) A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**, 265–267.

Anderson, L.D. & Atkins, E.L. (1968) Pesticide usage in relation to beekeeping. *Annual Review of Entomology* **13**, 213–238.

Atkins, E.L., Kellum, D. & Atkins, K.W. (1981) Reducing pesticide hazards to honey bees: mortality prediction techniques and integrated management strategies. Division of Agricultural Sciences, University of California. Leaflet #2883.

Brogdon, W.G. & McAllister, J.C. (1998) Simplification of adult mosquito bioassays through use of time-mortality determinations in glass bottles. *Journal of the American Mosquito Control Association* **14**, 159–164.

Caron, D.M. (1979) Effects of some ULV mosquito abatement insecticides on honey bees. *Journal of Economic Entomology* **72**, 148–151.

Chaskopoulou, A., Thrasyvoulou, A., Goras, G., Tananaki, C., Latham, M.D., Kashefi, J., Pereira, R.M. & Koehler, P.G. (2014) Nontarget effects of aerial mosquito adulticiding with water-based unsynergized pyrethroids on honey bees and other beneficial insects in an agricultural ecosystem of north Greece. *Journal of Medical Entomology* **51**, 720–724.

Colburn, R.B. & Langsford, G.S. (1970) Field evaluation of some mosquito adulticides with observations on toxicity to honey bees and house flies. *Mosquito News* **30**, 518–522.

Danka, R.G., Rinderer, T.E., Hellmich, R.L. & Collins, A.M. (1986) Comparative toxicities of four topically applied insecticides to Africanized and European honey bees (Hymenoptera: Apidae). *Journal of Economic Entomology* **79**, 18–21.

Dixon, D.P. & Fingler, B.G. (1984) *The Effects of the Mosquito Control Program on Bees Final Technical Report on Environmental Monitoring Program for the 1983 Spraying of Malathion to Combat Western Equine Encephalitis*. Winnipeg, Manitoba, Government of Manitoba.

Faraji, A., Unlu, I., Crepeau, T., Healy, S., Crans, S., Lizarraga, G., Fonseca, D. & Gaugler, R. (2016) Droplet characterization and penetration of an ultra-low volume mosquito adulticide spray targeting the Asian tiger mosquito, Aedes albopictus, within urban and suburban environments of Northeastern USA. *PLoS ONE* **11**, e0152069.

Fritz, B.K., Hoffman, W.C. & Jank, P. (2011) A fluorescent tracer method for evaluating spray transport and fate of field and laboratory spray applications. *Journal of ASTM* **8**, 1–9.

Gerberg, E.J., Barnard, D.R. & Ward, R.A. (1994) *Manual for Mosquito Rearing and Experimental Techniques Lake Charles*, La., American Mosquito Control Association.

Hardstone, M.C. & Scott, J.G. (2010) Is *Apis mellifera* more sensitive to insecticides than other insects? *Pest Management Science* **66**, 1171–1180.

Henry, M., Beguin, M., Requier, F., Rollin, O., Odoux, J.F., Aupinel, P., Aptel, J., Tchamitchian, S. & Decourtye, A. (2012) A common pesticide decreases foraging success and survival in honey bees. *Science* **336**, 348–350.

Hester, P.G., Shaffer, K.R., Tietze, N.S., Zhong, H. & Griggs, N.L. (2001) Efficacy of ground-applied ultra-low-volume malathion on honey bee survival and productivity in open and forest areas. *Journal of the American Mosquito Control Association* **17**, 2–7.

Iwasa, T., Motoyama, N., Ambrose, J.T. & Roe, M. (2004) Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. *Crop Protection* **23**, 371–378.

Johnson, R.M., Wen, Z., Schuler, M.A. & Berenbaum, M.R. (2006) Mediation of pyrethroid insecticide toxicity to honey

bees (Hymenoptera: Apidae) by cytochrome P450 mono-oxygenases. *Journal of Medical Entomology* **99**, 1046–1050.

Johnson, R.M., Pollock, H.S. & Berenbaum, M.R. (2009) Synergistic interactions between in-hive miticides in *Apis mellifera*. *Journal of Medical Entomology* **102**, 474–479.

Johnson, R.M., Dahlgren, L., Siegfried, B.D. & Ellis, M.D. (2013) Acaricide, fungicide and drug interactions in honey bees (*Apis mellifera*). *PLoS ONE* **8**, e54092.

Laurino, D., Manino, A., Patetta, A. & Parparato, M. (2013) Toxicity of neonicotinoid insecticides on different honey bee genotypes. *Bulletin of Insectology* **66**, 119–126.

Long, E.Y. & Krupke, C.H. (2016) Non-cultivated plants present a season-long route of pesticide exposure for honey bees. *Nature Communications* **7**.

Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., vanEngelsdorp, D. & Pettis, J.S. (2010) High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *PLoS ONE* **5**, e9754.

Pankiw, T. & Jay, S.C. (1992) Aerially applied ultra-low-volume malathion effects on caged honey bees (Hymenoptera: Apidae), caged mosquitoes (Diptera: Culicidae), and malathion residues. *Journal of Economic Entomology* **85**, 687–691.

Peterson, R.K., Preftakes, C.J., Bodin, J.L., Brown, C.R., Piccolomini, A.M. & Schleier, J.J. (2016) Determinants of

acute mortality of *Hippodamia convergens* (Coleoptera: Coccinellidae) to ultra-low volume permethrin used for mosquito management. *PeerJ* **4**, e2167.

Rinkevich, F.D., Margotta, J.W., Pittman, J.M., Danka, R.G., Tarver, M.R., Ottea, J.A. & Healy, K.B. (2015) Genetics, synergists, and age affect insecticide sensitivity in the honey bee, *Apis mellifera*. *PLoS ONE* **10**, e0139841.

Schleier, J.J. III & Peterson, R.K. (2010) Toxicity and risk of permethrin and naled to non-target insects after adult mosquito management. *Ecotoxicology* **19**, 1140–1146.

Schleier, J.J., Peterson, R.K., Irvine, K.M., Marshall, L.M., Weaver, D.K. & Preftakes, C.J. (2012) Environmental fate model for ultra-low-volume insecticide applications used for adult mosquito management. *Science of the Total Environment* **438**, 72–79.

Seccacini, E., Lucia, A., Harburguer, L., Zerba, E., Licastro, S. & Masuh, H. (2008) Effectiveness of pyriproxyfen and diflubenzuron formulations as larvicides against Aedes aegypti. *Journal of the American Mosquito Control Association* **24**, 398–403.

Zhong, H., Latham, M., Hester, P.G., Frommer, R.L. & Brock, C. (2003) Impact of naled on honey bee *Apis mellifera* L. survival and productivity: aerial ULV application using a flat-fan nozzle system. *Archives of Environmental Contamination and Toxicology* **45**, 216–220.