Journal of Medical Entomology, XX(X), 2020, 1–11
doi: 10.1093/jme/tjaa236
Research

Vector Control, Pest Management, Resistance, Repellents

NEVBD Pesticide Resistance Monitoring Network: Establishing a Centralized Network to Increase Regional Capacity for Pesticide Resistance Detection and Monitoring

James C. Burtis,[1,17] Joseph D. Poggi,[1] Joseph R. McMillan,[2] Scott C. Crans,[3] Scott R. Campbell,[4] Amy Isenberg,[5] Janice Pulver,[6] Patti Casey,[7] Kerry White,[8] Craig Zondag,[9] John R. Badger,[10] Russell Berger,[11] John Betz,[12] Stacey Giordano,[13] Malgorzata Kawalkowski,[14] John L. Petersen,[15] Gregory Williams,[16] Theodore G. Andreadis,[2] Philip M. Armstrong,[2] and Laura C. Harrington[1]

[1]Department of Entomology, Cornell University, Ithaca, NY 14850,[2]Connecticut Agricultural Experiment Station, New Haven, CT 06511,[3]NJDEP Office of Mosquito Control Coordination, Trenton, NJ 08625,[4]Suffolk County Department of Health Services, Yaphank, NY 11980,[5]Rockland County Department of Health, Pomona, NY 10970,[6]York County Mosquito Control, Yorktown, VA 23692,[7]Vermont Agency of Agriculture, Food & Markets, Montpelier, VT 05602,[8]BLSG Insect Control District, Brandon, VT 05733,[9]Lemon Fair Insect Control District, Weybridge, VT 05753,[10]Delaware Division of Fish and Wildlife, Mosquito Control Section, Milford, DE 19963,[11]Morris County Division of Mosquito Control, Morristown, NJ 07963,[12]Department of Public Works, Cumberland County Mosquito Control, Bridgeton, NJ 08302,[13]Warren County Mosquito Commission, Oxford, NJ 07863,[14]Suffolk County Department of Public Works, Vector Control, Yaphank, NY 11980,[15]Center for Vector Biology, Rutgers University, New Brunswick, NJ 08901,[16]Hudson Regional Health Commission, Secaucus, NJ 07094, and [17]Corresponding author, e-mail: burtis.james@gmail.com

Subject Editor: Ary Faraji

Received 12 June 2020; Editorial decision 25 September 2020

Abstract

Pesticide resistance in arthropod vectors of disease agents is a growing issue globally. Despite the importance of resistance monitoring to inform mosquito control programs, no regional monitoring programs exist in the United States. The Northeastern Regional Center for Excellence in Vector-Borne Diseases (NEVBD) is a consortium of researchers and public health practitioners with a primary goal of supporting regional vector control activities. NEVBD initiated a pesticide resistance monitoring program to detect resistant mosquito populations throughout the northeastern United States. A regionwide survey was distributed to vector control agencies to determine needs and refine program development and in response, a specimen submission system was established, allowing agencies to submit *Culex pipiens* (L.) (Diptera:Culicidae) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) for pesticide resistance testing. NEVBD also established larvicide resistance diagnostics for *Bacillus thuringiensis israelensis* (*Bti*) and methoprene. Additional diagnostics were developed for *Cx. pipiens* resistance to *Lysinibacillus sphaericus*. We received 58 survey responses, representing at least one agency from each of the 13 northeastern U.S. states. Results indicated that larvicides were deployed more frequently than adulticides, but rarely paired with resistance monitoring. Over 18,000 mosquitoes were tested from six states. Widespread low-level (1 × LC-99) methoprene resistance was detected in *Cx. pipiens*, but not in *Ae. albopictus*. No resistance to *Bti* or *L. sphaericus* was detected. Resistance to pyrethroids was detected in many locations for both species. Our results highlight the need for increased pesticide resistance testing in the United States and we provide guidance for building a centralized pesticide resistance testing program.

Key words: pesticide resistance, insecticide, *Aedes*, *Culex*, bioassay

Pesticide resistance affects mosquito populations across the globe, with the potential to hamper vector control efforts and attempts to reduce human cases of mosquito borne disease. A major issue is that only a few classes of pesticides are available for use in vector control and new classes are rarely developed. Since dichlorodiphenyl-trichloroethane (DDT) was introduced for vector control in 1946, synthetic pesticides have been widely used to control mosquitoes in the United States (Hemingway and Ranson 2000). Most pesticide classes currently deployed for mosquito control were developed decades ago. Recently, WHO (2017) recommended the first new pesticide class in 30 yr, chlorfenapyr, for targeted malaria vector control. Even this chemical was repurposed from urban and agricultural pest control, where it has been used since 1995. Resistance to many pesticide classes has been detected in mosquito field populations, including organophosphates (Lima et al. 2003), pyrethroids (Liu et al. 2006, Ranson et al. 2011), juvenile hormone regulators (Cornel et al. 2000, Vasquez et al. 2009), and some biopesticides (Rao et al. 1995). Mosquito control failures have also been linked with localized pesticide resistance in mosquito populations (Brouqui et al. 2012), including Brazil (Maciel-de-Freitas et al. 2014, Macoris et al. 2018), the United States (Estep et al. 2018), and many countries across Africa (Ranson and Lissenden 2016). Despite the limited number of active ingredients available and their importance in protecting public health, pesticide resistance monitoring is rarely conducted alongside control operations to ensure the long-term efficacy of available products.

The timeframe for the emergence of pesticide resistance in a mosquito population can vary depending on the climate, active ingredient deployed, application frequency, and genetic variation within the target mosquito population (Dusfour et al. 2019). The spatial scale of resistance hotspots can also vary, depending on geographical factors affecting connectivity between mosquito populations (Deming et al. 2016, Raghavendra et al. 2017). Furthermore, many public health pesticides are also used for agricultural pest control, so the susceptibility of mosquito populations may not be directly linked to pesticide deployment by vector control operations but rather the result of agricultural use (Chareonviriyaphap et al. 1999, Reid and McKenzie 2016). The spatiotemporal variation in the emergence of pesticide resistance in mosquitoes makes it difficult to predict when and where it will appear. Therefore, consistent monitoring of pesticide resistance in field collected specimens is necessary to provide control operators with information about the susceptibility of their target mosquito populations and the efficacy of the pesticides they deploy.

The United States has hundreds of state- and county-level mosquito control operations, which apply pesticides to control vector and nuisance mosquitoes. In a recent survey of mosquito control agencies by the National Association of County and City Health Officials (NACCHO), 98% of respondents identified pesticide resistance as a core competency that needs improvement (NACCHO 2017). Bioassay guidelines and materials for monitoring adulticide resistance are available (in the form of 'kits') from both the U.S. Centers for Disease Control and Prevention (CDC; McAllister and Scott 2019) and the World Health Organization (WHO) (WHO 2016a,b). The resistance monitoring kits include requisite materials to conduct bioassays, but laboratory space and training are more difficult to distribute to local jurisdictions. Furthermore, while guidelines exist, kits are not available for testing resistance to larvicides.

Comprehensive pesticide resistance networks can be highly effective. For example, the Superintendência de Controle de Endemias (SUCEN) in São Paulo, Brazil, initiated a centralized pesticide resistance program in 1996, which was expanded nationally in 1999 (Macoris et al. 2005). Through this program, moderate resistance to temephos in localized *Aedes Aegypti* (L.) (Diptera:Culicidae) populations was detected in initial surveys (Macoris et al. 2003) and was tracked as it emerged nationally over the following decade (Chediak et al. 2016). These data were used by the Brazilian Ministry of Health and their National Dengue Control Program to alter mosquito management methods and have allowed for the development of localized resistance management plans in Brazilian communities (Garcia et al. 2018).

Pesticide resistance monitoring for mosquitoes in the United States is often conducted by academic institutions (McAbee et al. 2004, Marcombe et al. 2014). It is also often targeted to test baseline resistance across multiple pesticide active ingredients at coarse geographical scales (Richards et al. 2017), or at the state level (Parker et al 2020). These efforts may not reflect the goals of the local mosquito control agencies. Furthermore, bioassay results can be affected by multiple factors, including temperature, mosquito body condition, life stage, adult sex, and age (Aïzoun et al. 2014, Glunt et al. 2014, Xu et al. 2014, Owusu et al. 2017), which can vary between studies. Variation in bioassay methods can make it difficult to use the results to inform mosquito control operations. Furthermore, the ad hoc nature of these studies may prevent detection of regional patterns in pesticide resistance. The centralization of resistance monitoring can also help standardize laboratory protocols and make it easier for resistance data to be shared between agencies to identify regional resistance trends and hotspots.

In the United States, CDC identified the need to increase partnership and collaboration between regional academic institutions and local vector control operations. In response, five regional Centers for Excellence were created to build and enhance these collaborative networks. The Northeast Regional Center for Excellence in Vector-Borne Diseases (NEVBD) initiated a pesticide resistance monitoring program with the goal of improving and expanding resistance operations in the northeastern United States. In 2019, NEVBD distributed a survey to the community of practice to identify needs, barriers to conducting monitoring, and knowledge gaps. Using this information, target species and pesticides were selected, larvicide bioassay guidelines developed, and a specimen submission system established. The submission system allowed collaborators working in state and local agencies to submit mosquitoes for testing to the NEVBD pesticide resistance monitoring laboratory at Cornell University, Ithaca, NY. The results of the NEVBD survey and resistance testing during the 2019 field season are presented here.

Methods

Pesticide Use and Resistance Survey

The goal of this survey was to determine the current capacity of regional resistance monitoring programs and to identify barriers for conducting resistance monitoring. A Qualtrics platform was used to create a draft survey that was initially tested among NEVBD partners. In addition, the survey was reviewed and approved by Cornell University's Institutional Review Board (protocol ID 1811008443) prior to dissemination. In total, 64 questions were divided into four sections, 1) participating agency information (Q1–Q7), 2) mosquito and tick surveillance (Q8–Q19), 3) mosquito and tick control (Q20–Q44), and 4) pesticide resistance monitoring (Q44–Q64) (Fig. 1). All responses were anonymous. Survey links were distributed in February 2019 to potential participants through the NEVBD and Northeastern Mosquito Control Association (NMCA) listservs, in addition to Twitter.

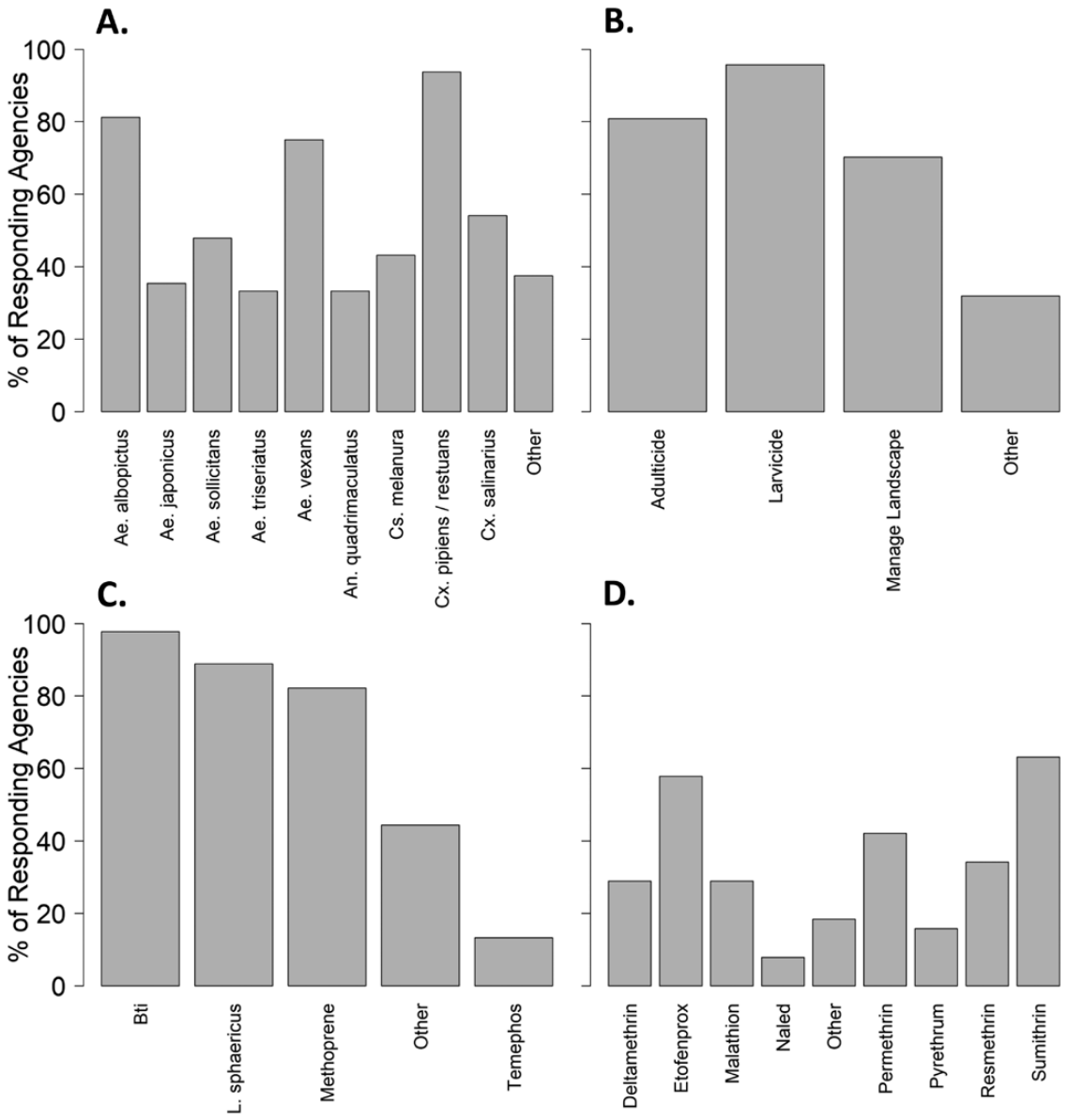

Fig. 1. Percent of responding agencies for the following questions: (A) what species of mosquitoes are the primary targets of your agency's control operations ($n = 48$), (B) does your agency commonly engage in any of the mosquito control procedures listed below ($n = 47$), (C) what larvicide active ingredients are used by your agency ($n = 45$), and (D) what adulticide active ingredients are used by your agency ($n = 38$). Each survey question allowed agency respondents to select multiple answers.

Mosquito Rearing

Based on information from the survey regarding target species, two susceptible mosquito strains were used to construct susceptibility curves, upon which larvicide resistance diagnostics were based. A susceptible *Aedes albopictus* (Skuse) (Diptera: Culicidae) colony was established from specimens collected in 2017 on Hahajima Island, an isolated Pacific island 950 km off the coast of Tokyo, Japan. A susceptible *Culex pipiens* (L.) (Diptera:Culicidae) colony was established by the San Mateo Mosquito and Vector Control District (Burlingame, CA) from specimens collected in Santa Clara, CA, in 2010. Su et al. (2018, 2019) provide detailed susceptibility profiles of the *Cx. pipiens* colony.

Field-collected mosquitoes and susceptible laboratory strains were maintained at 28° (±1) C and 80% (±10%) RH with a 12:12 (L:D) h cycle. After hatching, 200 larvae were transferred into trays containing 1 liter of distilled water. Larval food slurry containing fish food pellets (Cichlid Gold, Kyorin Co. LTD, Teaneck, NJ), rabbit pellets (FortiDiet Pro Health, Kaytee, Chilton, WI), and liver powder (MP Biomedicals Inc, Solon, OH) (30 ml, 1:2:1 mix of fish pellets:rabbit pellets:liver powder) was added to *Cx. pipiens* trays. *Aedes albopictus* larvae were fed four fish food pellets added to each tray. After pupation, mosquitoes were transferred to 30 cm^3 rearing cages or 2-liter buckets for adult eclosion. Both species were supplied with a 10% sugar solution. Females of both species were offered a bloodmeal from live restrained chickens placed inside cages for <20 min. All procedures involving chickens were approved by the Cornell University Institutional Animal Care and Use Committee (protocol ID 2001-0056).

Construction of Larvicide Diagnostics

Technical grade methoprene, mix of isomers, 95% purity, (Chem Service, West Chester, PA) was used in larval bioassays. Formulated products were used for biopesticides, an aqueous formulation of *Bacillus thuringiensis israelensis* (referred to as *Bti* hereafter; Valent Biosciences LLC, Vectobac 12AS, Libertyville, IL, 1,200 ITU/mg), and a granular formulation of *Lysinibacillus sphaericus* (Valent Biosciences LLC, VectoLex FG, 50 ITU/mg) were used to construct susceptibility curves. The same pesticide stocks were used for the larvicide resistance bioassays described below. *Bti* and *L. sphaericus* formulations were stored at room temperature (21°C), and methoprene was stored at 4°C. Methoprene was diluted in acetone, while *Bti* and *L. sphaericus* were diluted with distilled or deionized water. Prior to dilution, the VectoLex FG was also ground into powder and sifted through a fine (0.13 mm) sieve to ensure an even suspension in water. Bioassay containers were constructed by placing an 89-ml wax-lined paper cup inside a 475-ml cup. Each small cup contained 74 ml of water (70 ml for *Bti*), 50 mg of fish food, and 15 fourth-instar larvae. Either 1 ml (methoprene/*L. sphaericus*) or 5 ml (*Bti*) of pesticide was added to each cup. The large cup was covered in fine mesh to capture any eclosed adults from the methoprene trials. Larval mortality was recorded after 24 h for *Bti* and *L. sphaericus*. Methoprene bioassays were checked every 24 h until no living pupae remained and then the number of eclosed adults was recorded. To be counted, adults must have fully emerged from their pupal casing and both dead and alive adults were counted as eclosed.

The range of concentrations included for each susceptibility curve and number of replicates divided by active ingredient and mosquito species tested are presented in Table 1. No *L. sphaericus* curve was constructed for *Ae. albopictus*. Negative controls with DI water (*L. sphaericus* / *Bti*) or acetone (methoprene) were conducted alongside all trials. If control mortality was >10%, data were discarded. Control mortality was adjusted with Abbott's correction (Abbott 1925) for each trial. A trial consisted of no more than 10 bioassay cups, all prepared at the same time along with their controls.

Collection of Field Specimens

Mosquitoes were collected from the field in the egg stage and submitted using species-specific collection kits supplied by NEVBD. *Culex pipiens* egg rafts were collected from field locations and hatched. First and second instar larvae were packed into whirlpak bags (Nasco, Atkinson, WI), placed on ice inside an insulated container and shipped to Cornell University. *Culex pipiens* were identified as larvae upon arrival using a taxonomic key (Andreadis et al. 2005). *Aedes albopictus* eggs were collected from the field on paper towel sheets clipped to the inside of black water-holding buckets. Once egg sheets were collected, papers were dried, packed inside a sealable plastic bag and shipped. Egg sheets were stored at 28°C in an incubator for <2 mo before vacuum hatching. *Aedes albopictus* egg sheets were examined for species-specific chorion sculpturing under a dissection scope at 40× magnification (Shragai et al. 2018). Any non *Ae. albopictus* eggs were destroyed by poking with a pin prior to hatching. A subset of hatched specimens also were identified using a taxonomic key (Andreadis et al. 2005). Only mosquitoes hatched from field collected eggs (F0) were reared as described above and tested for resistance in bioassays.

Larvicide Resistance Bioassays

Larvicide resistance bioassays were conducted with the same containers and negative controls (acetone/water) as those described above for constructing the diagnostic curves. To ensure that stock solutions remained viable, trials were conducted with susceptible

Table 1. The result of probit analyses for the five susceptibility curves that were constructed to determine the diagnostic doses for *Aedes albopictus* and *Culex pipiens*

Pesticide	Species	Concentration range	Replicates/concentration	df	Slope (SE)	LC-50 (± 95% CI)	LC-95 (± 95% CI)	LC-99 (± 95% CI)	χ^2
Methoprene	*Aedes albopictus*	0.002–0.2 (µg/ml)	10	148	2.4 (0.1)	0.0092 (±0.00079)	0.044 (±0.0072)	0.085 (±0.016)	489
	Culex pipiens	0.00003–0.007 (µg/ml)	15	124	1.2 (0.1)	0.00014 (±0.000022)	0.0035 (±0.0012)	0.013 (±0.0044)	387
Bti	*Aedes albopictus*	0.0002–0.04 (ITU/ml)	11	96	1.7 (0.08)	0.0026 (±0.00030)	0.023 (±0.0064)	0.058 (±0.016)	440
	Culex pipiens	0.0002–0.02 (ITU/ml)	12	94	2.1 (0.09)	0.0012 (±0.00011)	0.0073 (±0.0015)	0.015 (±0.0033)	493
Lysinibacillus sphaericus	*Culex pipiens*	0.002–0.3 (ITU/ml)	12	93	1.7 (0.08)	0.017 (±0.0018)	0.14 (±0.035)	0.35 (±0.12)	474

The units for the LC-50, LC-95, and LC-99 values are µg/ml.

mosquitoes at LC-95 values regularly to test the efficacy of each active ingredient. If mortality was less than 90% the stock was discarded and remixed. Only one methoprene stock was discarded after it had been stored in acetone for ~4 mo. Data from all bioassays using that stock were also discarded. All trials were conducted under the rearing conditions described above.

The level of resistance for methoprene, *bti*, and *L. sphaericus* was scaled (low/moderate/high). If less than 90% mortality was observed at the LC-99 value, the population was classified with 'low-level resistance'. If low-level resistance was detected, and specimens were available, 'moderate resistance' (LC-99 × 5) and 'high-level resistance' (LC-99 × 10) trials were conducted for that active ingredient. The thresholds for moderate and high-level resistance were also less than 90% mortality at those respective concentrations (WHO 2016b). The same scale was used for *L. sphaericus* using the LC-95 value.

Adulticide Resistance Bioassays

The CDC bottle bioassay method was used to test mosquitoes for resistance to adulticides (McAllister and Scott 2019). All adulticide active ingredients were technical grade and supplied directly by the Division of Vector-Borne Diseases CDC. Wheaton bottles (250 ml) were treated by adding 1 ml of active ingredient in acetone at the diagnostic dose to each bottle. The concentrations provided in the kit were as follows: deltamethrin (0.75 µg/ml), etofenprox (12.5 µg/ml), malathion (400 µg/ml), permethrin (43 µg/ml), prallethrin (0.05 µg/ml), pyrethrum (15 µg/ml), and sumithrin (20 µg/ml). Bottles were inverted to treat the caps, and then rolled for at least 20 min with the caps removed. Control bottles were treated with acetone using the same procedure. Between 20 and 25 sugar-fed 2- to 6-d-old mated females were added to each bottle after treatment. All CDC bottle bioassays were conducted under the same environmental conditions as described for mosquito rearing above. If less than 90% mortality was observed at the diagnostic time, mosquitoes were classified as 'low-level' resistant. Less than 90% mortality at two times the diagnostic time was classified as 'moderate' resistance, and less than 90% mortality at three times the diagnostic time was 'high-level' resistance. All bioassays were continued for 120 min or until 100% mortality was reached. Trials with more than 10% control mortality were discarded, and Abbott's correction for control mortality was applied to the remaining trials.

Statistical Analyses

The diagnostic concentrations (LC-99 and LC-95) for larvicide resistance were determined using probit analysis, conducted in R using the glm() command in the MASS package (see Supp Materials [online only] for code). Resistance data for each pesticide tested were summarized at the town-level to remove detailed location identifiers as requested by our collaborators.

Results

Pesticide Use and Resistance Survey

In total, 58 agencies participated in the pesticide use and resistance survey between 4 February to 24 April 2019. Respondents participated from each of the 13 states in the NEVBD jurisdiction, in addition to Washington, DC. The number of respondents per state-level jurisdiction included the following: Connecticut (2), Delaware (1), Maine (1), Maryland (2), Massachusetts (8), New Hampshire (1), New Jersey (17), New York (5), Pennsylvania (6), Rhode Island (1), Vermont (3), Virginia (6), Washington, DC (1), West Virginia (1), and Other (3). Of the respondents, 41.7% worked at the state level, whereas 58.3% worked within local (county/city) jurisdictions. Four primary questions guided the selection of target mosquito species and pesticides for the NEVBD pesticide resistance monitoring program: 1) what species of mosquitoes are the primary targets of your agency's control operations, 2) does your agency commonly engage in any of the mosquito control procedures listed below, 3) what larvicide active ingredients are used by your agency, and 4) what adulticide active ingredients are used by your agency (Fig. 1).

Whereas 95.7% (45/47) of responding agencies deployed larvicides for mosquito control, only 6% (3/50) conducted regular monitoring for resistance to larvicide products. Moreover, 80.9% (38/47) of agencies deployed adulticides, and 31.4% (16/51) conducted regular monitoring of their mosquito populations for resistance to adulticides. Respondents were also asked to rank major limiting factors preventing their agencies from attempting to conduct both larvicide and adulticide resistance bioassays, with one being most limiting and five being least limiting. For adulticide resistance, factors limiting testing were ranked as follows: training (average score of 2.25), personnel (2.5), equipment (2.58), funding (2.67), and other (5). For larvicide resistance, factors were ranked as follows: training (2.37), funding (2.43), equipment (2.53), personnel (2.8), and other (4.87). Respondents were given the option to fill in additional details for the 'other' option on both questions, but none did so. The full survey report and data are available in the Supp Materials (online only).

Probit Analyses

Methoprene probit analyses for *Ae. albopictus* (t = 22.1; df = 148; $P < 0.001$) and *Cx. pipiens* (t = 19.7; df = 124; $P < 0.001$) were conducted to determine the diagnostic doses (LC-99) used to test field populations for resistance. The *Bti* probit analyses for *Ae. albopictus* (t = 21; df = 96; $P < 0.001$) and *Cx. pipiens* (t = 22.2; df =94; $P < 0.001$) were also conducted to determine the diagnostic doses (LC-99). To explore resistance further, a subsample of mosquitoes from Connecticut were tested at the LC-95 value from the *L. sphaericus* probit analysis for *Cx. pipiens* (t = 21.3; df =94; $P < 0.001$; Table 1; Fig. 2). For *Cx. pipiens*, the diagnostic doses used to test specimens from the field were 0.013 µg/ml for methoprene, 0.015 ITU/ml for *Bti*, and 0.14 ITU/ml for *L. sphaericus*. The diagnostic doses for *Ae. albopictus* were 0.085 µg/ml for methoprene and 0.058 ITU/ml for *Bti*.

Resistance to Larvicides and Adulticides

In total, 1,416 *Ae. albopictus* and 13,200 *Cx. pipiens* specimens were tested for resistance to larvicides (methoprene/*Bti*/*L. sphaericus*; Table 2). Widespread low-level (LC-99 × 1) methoprene resistance was detected in multiple *Cx. pipiens* populations across the northeastern United States (Fig. 3). No methoprene resistance was detected in *Ae. albopictus* populations. No resistance was detected to either *Bti* or *L. sphaericus* in either species.

For adulticide resistance, 910 *Ae. albopictus* and 2,990 *Cx. pipiens* were tested. Bioassay tests were conducted for five pyrethroid active ingredients: deltamethrin, etofenprox, permethrin, prallethrin, and sumithrin. In addition, adult populations were tested for resistance to one pyrethrin (pyrethrum) and an organophosphate (malathion). Adult resistance to pyrethroids was detected in *Ae. albopictus* and *Cx. pipiens* populations at varying levels. Resistance was detected in *Ae. albopictus* populations to etofenprox (one location), permethrin (two locations), and sumithrin (one location). Resistance was detected in *Cx. pipiens* to deltamethrin (two locations), etofenprox (two locations), permethrin (two locations), and

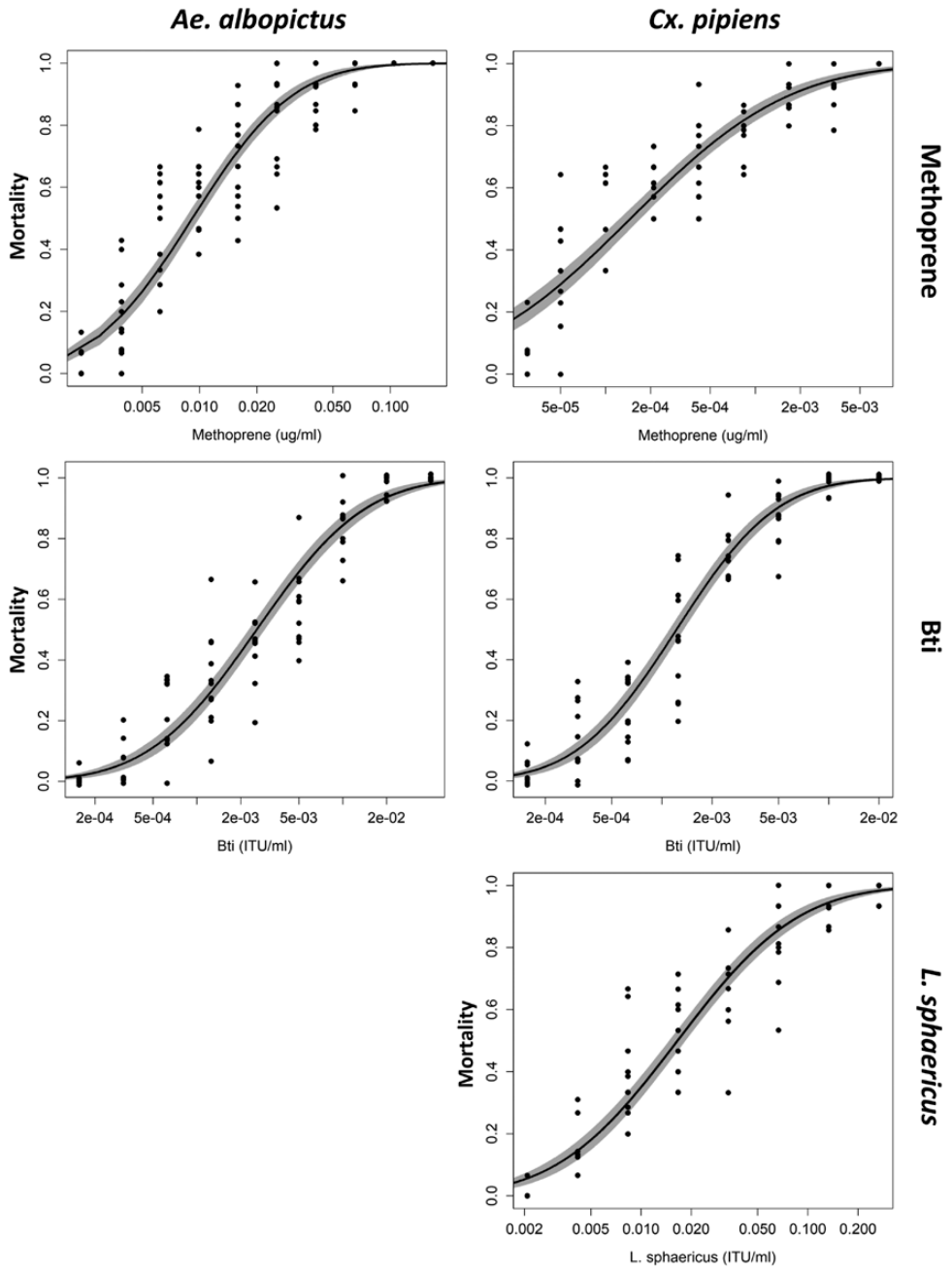

Fig. 2. Scatterplots showing the five susceptibility curves for the three larvicide active ingredients (Bti/ *Lysinibacillus sphaericus*/methoprene) and two species (*Aedes albopictus*/*Culex pipiens*). The lines represent the prediction resulting from probit analyses and the shaded areas are the 95% CI of the prediction. A jitter was used to reveal overlapping points.

sumithrin (three locations). Most notably, *Cx. pipiens* populations in Virginia were resistant to several pyrethroid active ingredients, including deltamethrin, etofenprox, permethrin, and sumithrin. *Culex pipiens* collected from Virginia demonstrated moderate pyrethrum resistance. No malathion resistance was detected in the three *Cx. pipiens* populations tested (Table 3).

Discussion

Results from our pesticide use and resistance survey showed that larviciding was the most common control method deployed in the northeastern United States, but that monitoring for resistance to larvicides was rare. Only 6% of agencies conducted regular testing for larvicide resistance, whereas 31.4% conducted regular monitoring for resistance to adulticides. Training, funding, and time were all major limiting factors for agencies attempting to conduct both larvicide and adulticide resistance bioassays. As a result, NEVBD developed and distributed specimen collection kits and guidelines to potential collaborators throughout the region to support the specimen submission system. NEVBD also developed larvicide resistance bioassay diagnostics. The primary objective of this system was to reduce the effort required for mosquito control operations in the northeastern United States to monitor pesticide resistance in their jurisdictions.

Table 2. Larval bioassay results for *Aedes albopictus* and *Culex pipiens* specimens submitted by field collaborators in 2019

Species	Active ingredient	State	Town	Number of collection locations	Number of mosquitoes tested	% Mortality	Level of resistance
Aedes albopictus	*Bti*	DE	Millsboro	1	153	100.0	None
Aedes albopictus	*Bti*	NY	Stony Brook	2	363	100.0	None
Aedes albopictus	*Bti*	NY	Valley Cottage	1	149	99.3	None
Aedes albopictus	Methoprene	DE	Milford	1	151	96.7	None
Aedes albopictus	Methoprene	NY	Stony Brook	3	450	98.2	None
Aedes albopictus	Methoprene	NY	Valley Cottage	1	150	98.7	None
Culex pipiens	Methoprene	CT	Milford	10	1484	79.3	Low
Culex pipiens	Methoprene (LC-99 × 5)	CT	Milford	6	600	100.0	
Culex pipiens	Methoprene	CT	New Haven	3	452	42.0	Moderate
Culex pipiens	Methoprene (LC-99 × 5)	CT	New Haven	2	300	86.7	
Culex pipiens	Methoprene	CT	Stratford	3	417	48.7	Low
Culex pipiens	Methoprene (LC-99 × 5)	CT	Stratford	1	150	99.3	
Culex pipiens	Methoprene	DE	Milford	1	150	78.0	Low
Culex pipiens	Methoprene (LC-99 × 5)	DE	Milford	1	150	100.0	
Culex pipiens	Methoprene	NY	Liverpool	2	300	62.0	Low
Culex pipiens	Methoprene (LC-99 × 5)	NY	Liverpool	2	300	99.3	
Culex pipiens	Methoprene	NY	Nedrow	1	150	84.0	Low
Culex pipiens	Methoprene	NY	Pomona	1	150	30.7	Low
Culex pipiens	Methoprene (LC-99 × 5)	NY	Pomona	1	120	97.5	
Culex pipiens	Methoprene	NY	Stony Point	1	90	58.9	Low
Culex pipiens	Methoprene	NY	Syracuse	1	150	55.3	Low
Culex pipiens	Methoprene	VT	Salisbury	1	150	100.0	None
Culex pipiens	Methoprene	VT	Weybridge	3	450	100.0	None
Culex pipiens	Methoprene	VA	Williamsburg	2	300	37.7	Low
Culex pipiens	Methoprene (LC-99 × 5)	VA	Williamsburg	1	150	91.3	
Culex pipiens	*Bti*	CT	Milford	3	453	99.7	None
Culex pipiens	*Bti*	CT	New Haven	2	300	99.0	None
Culex pipiens	*Bti*	CT	Stratford	5	724	99.7	None
Culex pipiens	*Bti*	DE	Milford	2	224	100.0	None
Culex pipiens	*Bti*	DE	Millsboro	1	153	100.0	None
Culex pipiens	*Bti*	NY	Liverpool	2	300	100.0	None
Culex pipiens	*Bti*	NY	Nedrow	2	241	100.0	None
Culex pipiens	*Bti*	NY	Syracuse	1	150	100.0	None
Culex pipiens	*Bti*	VT	Salisbury	1	151	99.3	None
Culex pipiens	*Bti*	VT	Weybridge	2	302	99.3	None
Culex pipiens	*Bti*	VA	Williamsburg	1	150	100.0	None
Culex pipiens	*Lysinibacillus sphaericus*	CT	Milford	14	2097	99.8	None
Culex pipiens	*Lysinibacillus sphaericus*	CT	New Haven	2	300	99.0	None
Culex pipiens	*Lysinibacillus sphaericus*	CT	Stratford	9	1346	99.1	None
Culex pipiens	*Lysinibacillus sphaericus*	NY	Liverpool	1	147	100.0	None
Culex pipiens	*Lysinibacillus sphaericus*	NY	Syracuse	1	149	100.0	None

Results are collated at the town-level. Percent mortality represents the average mortality of all field collections within that administrative boundary. All bioassays listed below are tests for low-level resistance, unless otherwise stated.

The NEVBD specimen submission system allowed for the detection of wide-spread low-level resistance to methoprene in *Cx. pipiens* populations throughout the northeastern United States. We only identified one population in Connecticut with moderate resistance (LC-99 × 5), which coincides with the only vector control program in that state that regularly deploys methoprene. Notably, methoprene resistance was detected in localities where mosquito control agencies did not regularly use that active ingredient. This was the case for the low-level resistance observed in the Williamsburg area of York County, VA, which nearly approached the threshold for moderate resistance with 91.3% mortality at the LC-99 × 5 value. Methoprene is commonly used in the southern

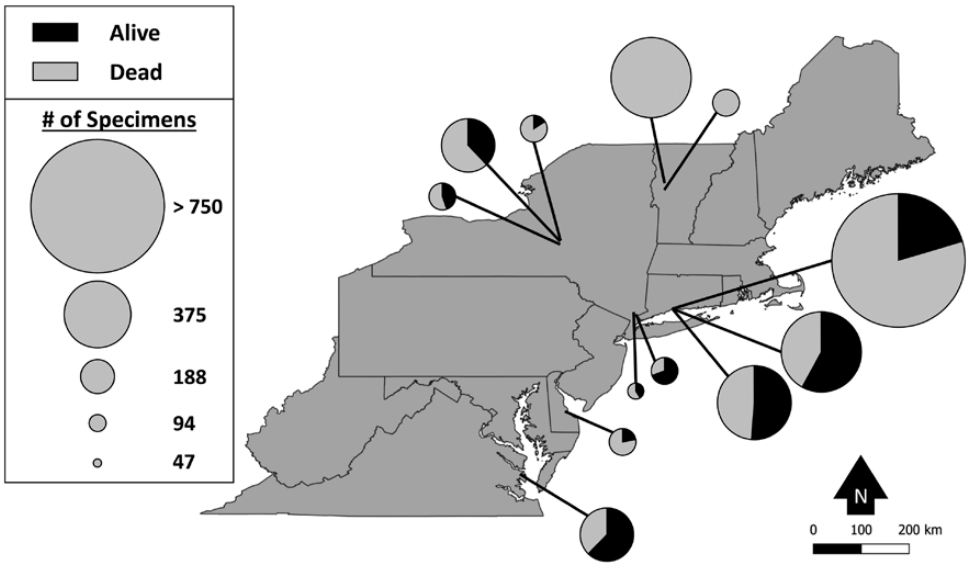

Fig. 3. Results of the bioassay tests for low-level (LC-99 × 1) methoprene resistance for *Cx pipiens*. Pie charts show the percent mortality and the size of each pie chart relates to the number of specimens tested from each town.

part of York County, but not as often in the Williamsburg area where samples were collected and we detected low-level resistance (J. Pulver, personal communication). More study is needed between the Northern and Southern parts of York County, as well as the broader region, to determine the geographical extent of the resistant population. Additionally, the level of agricultural and personal use of methoprene is unknown. Methoprene is commonly used for flea control and control of insects in stored food products and may further contribute to resistance as agricultural and residential use of pesticides can outweigh the amount used for vector control (Reid and McKenzie 2016). Methoprene resistance has previously been reported in Florida (Marcombe et al. 2014) and New York (Paul et al. 2005), but not at a regional scale. It is also worth noting that we counted the number of adults that fully eclosed, whether dead or alive in our resistance bioassays. Resistance is defined by a threshold determined using a susceptible colony. Our baseline data

Table 3. CDC bottle bioassay results for *Aedes albopictus* and *Culex pipiens* specimens submitted by collaborators from the field in 2019

Species	Active ingredient	State	Town	% Mortality (diagnostic time)	Time to 100% mortality	Number of mosquitoes tested	Level of resistance
Aedes albopictus	Etofenprox	NJ	Cedar Knolls	95.2 (30 min)	45	145	None
Aedes albopictus	Etofenprox	NJ	Phillipsburg	10.5 (30 min)	105	153	High
Aedes albopictus	Permethrin	NJ	Flemington	77.1 (10 min)	15	158	Low
Aedes albopictus	Permethrin	NJ	Vineland	51 (10 min)	15	98	Low
Aedes albopictus	Sumithrin	NJ	Cedar Knolls	38.2 (45 min)	105	152	Moderate
Aedes albopictus	Sumithrin	NY	Lindenhurst	98.1 (45 min)	60	157	None
Aedes albopictus	Sumithrin	NY	Stony Brook	91.7 (45 min)	75	145	None
Culex pipiens	Deltamethrin	VA	Williamsburg	4.9 (45 min)	>120	155	High
Culex pipens	Deltamethrin	VA	York	0.5 (45 min)	>120	189	High
Culex pipiens	Etofenprox	VA	Williamsburg	64.1 (15 min)	105	156	High
Culex pipiens	Etofenprox	VA	York	0.7 (15 min)	105	144	High
Culex pipiens	Malathion	NJ	Secaucus	100.0 (45 min)	45	164	None
Culex pipiens	Malathion	VT	Salisbury	100.0 (45 min)	45	155	None
Culex pipiens	Malathion	VT	Weybridge	100.0 (45 min)	45	139	None
Culex pipiens	Permethrin	VT	Salisbury	100.0 (30 min)	30	179	None
Culex pipiens	Permethrin	VT	Weybridge	100.0 (30 min)	30	174	None
Culex pipiens	Permethrin	VA	Williamsburg	61.1 (30 min)	75	167	Moderate
Culex pipiens	Permethrin	VA	York	62.6 (30 min)	45	147	Low
Culex pipiens	Prallethrin	VA	Williamsburg	100.0 (60 min)	60	176	None
Culex pipiens	Pyrethrum	VA	Williamsburg	92.3 (45 min)	>120	130	None
Culex pipiens	Pyrethrum	VA	York	63.5 (45 min)	>120	178	High
Culex pipiens	Sumithrin	CT	New Haven	96.6 (30 min)	45	89	None
Culex pipiens	Sumithrin	CT	New Haven	27.3 (30 min)	>120	139	High
Culex pipiens	Sumithrin	CT	Fairfield	94.3 (30 min)	75	70	None
Culex pipiens	Sumithrin	VT	Salisbury	100.0 (30 min)	30	81	None
Culex pipiens	Sumithrin	VT	Weybridge	85.0 (30 min)	45	180	Low
Culex pipiens	Sumithrin	VA	Williamsburg	30.0 (30 min)	90	170	Moderate

were collected using the same method, and while useful for detecting resistance or reduced susceptibility in these populations, some mosquitoes exposed to methoprene applied for control in the field may eclose and die rapidly thereafter. As a consequence, our resistance bioassay results may be an underestimate of operational efficacy. There also may be sublethal effects which impact female longevity (Sawby et al. 1992), but additional research is needed to determine whether this effect varies by species and how it ultimately impacts field efficacy.

No resistance to either biopesticides, *Bti* and *L. sphaericus*, was detected in either species. With over 3,000 specimens tested for *Bti* resistance across the region, it is unlikely that resistance is emerging in the northeastern United States, despite its wide-spread deployment. This lack of resistance has been observed in other regions which primarily deploy *Bti* for mosquito control. Even in European mosquito populations that have been under relatively high selection pressure for long periods of time, resistance to *Bti* has not been detected (Becker et al. 2018). Resistance to *Bti* is rare because its mode of action involves multiple toxins, requiring resistance mechanisms to target these toxins for the phenotype to be expressed (Paris et al. 2011). Resistance to *Bti* has been detected in the United States (Paul et al. 2005), but upon resampling the same area in Nedrow, NY, in the current study, we did not detect *Bti* resistance. It is difficult to determine the cause of these conflicting results, but it is possible that the susceptible population recovered in the past 15 yr. It also may reflect changes in mosquito management intensity in the area. Conversely, resistance to *L. sphaericus* has been detected in multiple instances (Rao et al. 1995, Rodcharoen and Mulla 1996), and many formulations are used by mosquito control operations in our region. The focal area for testing this product was in coastal Connecticut, and while we did not detect resistance in these locations, it is possible that other populations in the northeastern United States are resistant to *L. sphaericus*-based products. Overall, increased monitoring for resistance to larvicides across the northeastern United States is needed, particularly given the dependence of many regional mosquito control operations on these products.

Resistance to a variety of pyrethroid active ingredients was detected throughout the region. The levels of resistance varied, with *Cx. pipiens* specimens from Virginia showing cross-resistance to multiple products. Pyrethroid resistance is common in the United States and abroad (Liu et al. 2006). Pyrethroid resistant *Ae. aegypti* populations have been detected in southern Florida. A highly resistant population was identified in Miami-Dade county in Florida (Estep et al. 2018). The detection of this resistant population informed the control methods deployed during a Zika outbreak in 2016 and may have allowed for the successful suppression of transmission (McAllister et al. 2020). This highlights the need to monitor pesticide resistance before an outbreak, allowing for appropriate control decisions to be made early in the event. We did not detect resistance to organophosphates, but specimens were only tested from three locations and only for one active ingredient, malathion. Potential emerging resistance to malathion was detected in *Ae. albopictus* populations in 2011 in New Jersey (Marcombe et al. 2014). Therefore, more widespread testing for resistance to organophosphates is necessary to determine the resistance status of mosquitoes in the northeastern region. Testing for resistance to organophosphates is particularly important, given the wide-spread resistance to pyrethroids detected. Currently, many states do not have organophosphate products registered for use for mosquito control. In the event of a public health emergency in the face of pyrethroid-resistant mosquitoes, organophosphates may be the most viable alternative for mosquito control operations in the northeastern United States. Therefore, we suggest continued testing of a wide variety of active ingredients, including those used in products that are not currently registered for vector control.

The wide-spread resistance we detected to methoprene and pyrethroids in the northeast United States highlights the need for increased surveillance in the United States, particularly given the broad use of these same active ingredients for vector control and agriculture. These monitoring efforts will be particularly important as new and reemerging mosquito-borne diseases increase in the United States, and the burden of endemic infections, including West Nile and eastern equine encephalitis, increase (Gage et al. 2008, Rosenberg et al. 2018). The increasing threat to public health from vector-borne diseases necessitates a well-organized and informed effort to ensure that the few tools available for vector control remain viable. Resources are often limited at the state and county level, so programs funded through CDC Centers of Excellence and academic institutions can help bolster these efforts. The NEVBD pesticide resistance monitoring system demonstrates that a centralized system, integrated with the local public health community of practice, can enhance the detection of pesticide resistance at a regional scale.

We acknowledge that our program structure may not be effective for every region in the United States, as institutions differ between states. In order for pesticide resistance monitoring programs to be successfully expanded, key barriers and challenges facing mosquito control districts must be addressed. The primary focus of centralized programs should be to provide support directly to local mosquito control districts, as large resource gaps exist between agencies. This support can be provided through targeted training opportunities and guidelines that are available to operators. Topics of importance include resistance testing methodology, interpretation, and application of resistance data to inform control operations, and methods for the rearing and collection of mosquitoes from the field. Ideally, these materials should be available in digital and live classroom formats to increase engagement. Due to the resource gaps that exist between agencies, particularly at the local-level, direct provision of funding for personnel or supplies can be impactful ways to assist smaller operations. A centralized system provides a consistent point of contact for operators interested in conducting resistance monitoring operations and reduces time, personnel, and equipment-related burdens on local and state mosquito control districts. These actions are necessary to bolster the national capability to monitor pesticide resistance in mosquito-borne disease vectors, allowing for the detection of resistance before it can contribute to control failures and negatively impact human health.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

Acknowledgments

We thank Dr. Janet McAllister of the United States Centers for Disease Control and Prevention for supplying the CDC bottle bioassay kits and for her guidance during the development of this program. Thank you to Dr. Steven Su, Scientific Programs Director at West Valley Mosquito Control District in California, for providing the susceptible *Culex pipiens* colony. We are grateful to Dr. Shinji Kasai, Director, Department of Medical Entomology at the National Institute of Infectious Diseases, Japan for providing the susceptible *Aedes albopictus* colony. Thank you to all the anonymous participants who completed the pesticide use and resistance survey. We would like to acknowledge the contribution of all staff in districts or agencies not mentioned by name who assisted us with the various aspects

of this project. Additionally, we would like to thank Sylvie Pitcher and Juan Pablo Jordan Davalos, along with the rest of the Harrington laboratory, for their extraordinary assistance collecting data during the field season. This work was supported through Cooperative Agreement U01CK000509 between the Centers for Disease Control and Prevention (CDC) and Cornell University/Northeast Regional Center for Excellence in Vector-Borne Diseases.

References Cited

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

Aïzoun, N., R. Aïkpon, R. Azondekon, A. Asidi, and M. Akogbéto. 2014. Comparative susceptibility to permethrin of two *Anopheles gambiae* sl populations from Southern Benin, regarding mosquito sex, physiological status, and mosquito age. Asian Pac. J. Trop. Biomed. 4: 312–317.

Andreadis, T. G., M. C. Thomas, and J. J. Shepard. 2005. Identification guide to the mosquitoes of Connecticut (No. 966). Connecticut Agricultural Experiment Station, New Haven, CT.

Becker, N., M. Ludwig, and T. Su. 2018. Lack of resistance in *Aedes vexans* field populations after 36 years of *Bacillus thuringiensis* subsp. *israelensis* applications in the Upper Rhine Valley, Germany. J. Am. Mosquito Contr. 34: 154–157.

Brouqui, P., P. Parola, and D. Raoult. 2012. Insecticide resistance in mosquitoes and failure of malaria control. Expert Rev. Anti. Infect. Ther. 10: 1379–1381.

Chareonviriyaphap, T., B. Aum-Aung, and S. Ratanatham. 1999. Current insecticide resistance patterns in mosquito vectors in Thailand. SE Asian J. Trop. Med. 30: 184–194.

Chediak, M., F. G Pimenta, Jr., G. E. Coelho, I. A. Braga, J. B. Lima, K. R. Cavalcante, L. C. Sousa, M. A. Melo-Santos, M. d. e. L. Macoris, A. P. Araújo, et al. 2016. Spatial and temporal country-wide survey of temephos resistance in Brazilian populations of *Aedes aegypti*. Mem. Inst. Oswaldo Cruz. 111: 311–321.

Cornel, A. J., M. A. Stanich, D. Farley, F. S. Mulligan 3rd, and G. Byde. 2000. Methoprene tolerance in *Aedes nigromaculis* in Fresno County, California. J. Am. Mosq. Control Assoc. 16: 223–228.

Deming, R., P. Manrique-Saide, A. Medina Barreiro, E. U. Cardeña, A. Che-Mendoza, B. Jones, K. Liebman, L. Vizcaino, G. Vazquez-Prokopec, and A. Lenhart. 2016. Spatial variation of insecticide resistance in the dengue vector *Aedes aegypti* presents unique vector control challenges. Parasit. Vectors. 9: 67.

Dusfour, I., J. Vontas, J. P. David, D. Weetman, D. M. Fonseca, V. Corbel, K. Raghavendra, M. B. Coulibaly, A. J. Martins, S. Kasai, et al. 2019. Management of insecticide resistance in the major *Aedes* vectors of arboviruses: advances and challenges. PLoS Neglect. Trop. Dis. 13: e0007615.

Estep, A. S., N. D. Sanscrainte, C. M. Waits, S. J., Bernard, A. M. Lloyd, K. J. Lucas, E. A. Buckner, R. Vaidyanathan, R. Morreale, L. A. Conti, et al. 2018. Quantification of permethrin resistance and kdr alleles in Florida strains of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse). PLoS Neglect. Trop. Dis. 12: e0006544.

Gage, K. L., T. R. Burkot, R. J. Eisen, and E. B. Hayes. 2008. Climate and vectorborne diseases. Am. J. Prev. Med. 35: 436–450.

Garcia, G. A., M. R. David, A. de Jesus Martins, R. Maciel-de-Freitas, J. G. B. Linss, S. C. Araújo, J. B. P. Lima, and D. Valle. 2018. The impact of insecticide applications on the dynamics of resistance: the case of four *Aedes aegypti* populations from different Brazilian regions. PLoS Neglect. Trop. D. 12: e0006227.

Glunt, K. D., K. P. Paaijmans, A. F. Read, and M. B. Thomas. 2014. Environmental temperatures significantly change the impact of insecticides measured using WHOPES protocols. Malaria J. 13: 350.

Hemingway, J., and H. Ranson. 2000. Insecticide resistance in insect vectors of human disease. Annu. Rev. Entomol. 45: 371–391.

Lima, J. B., M. P. Da-Cunha, R. C. Da Silva, A. K. Galardo, S. d. a. S. Soares, I. A. Braga, R. P. Ramos, and D. Valle. 2003. Resistance of *Aedes aegypti* to organophosphates in several municipalities in the State of Rio de Janeiro and Espírito Santo, Brazil. Am. J. Trop. Med. Hyg. 68: 329–333.

Liu, N., Q. Xu, F. Zhu, and L. E. E. Zhang. 2006. Pyrethroid resistance in mosquitoes. Insect Sci. 13: 159–166.

Maciel-de-Freitas, R., F. C. Avendanho, R. Santos, G. Sylvestre, S. C. Araújo, J. B. P. Lima, A. J. Martins, G. E. Coelho, and D. Valle. 2014. Undesirable consequences of insecticide resistance following *Aedes aegypti* control activities due to a dengue outbreak. PLoS One. 9: e92424.

Macoris, M. D. L. G., M. T. M. Andrighetti, L. Takaku, C. M. Glasser, V. C. Garbeloto, and J. E. Bracco. 2003. Resistance of *Aedes aegypti* from the state of São Paulo, Brazil, to organophosphates insecticides. Mem. Inst. Oswaldo Cruz. 98: 703–708.

Macoris, G., M. T. Macoris Andrighetti, K. D. C. Rodrigues Nalon, V. C. Garbeloto, and A. L. Caldas. 2005. Standardization of bioassays for monitoring resistance to insecticides in *Aedes aegypti*. Dengue Bull. 29: 176–182.

Macoris, M. L., A. J. Martins, M. T. M. Andrighetti, J. B. P. Lima, and D. Valle. 2018. Pyrethroid resistance persists after ten years without usage against *Aedes aegypti* in governmental campaigns: lessons from São Paulo State, Brazil. Plos Negl. Trop. Dis. 12: e0006390.

Marcombe, S., A. Farajollahi, S. P. Healy, G. G. Clark, and D. M. Fonseca. 2014. Insecticide resistance status of United States populations of *Aedes albopictus* and mechanisms involved. PLoS One. 9: e101992.

McAbee, R. D., K. D. Kang, M. A. Stanich, J. A. Christiansen, C. E. Wheelock, A. D. Inman, B. D. Hammock, and A. J. Cornel. 2004. Pyrethroid tolerance in *Culex pipiens pipiens* var *molestus* from Marin County, California. Pest Manag. Sci. 60: 359–368.

McAllister, J. C. and M. L. Scott. 2019. Centers for Disease Control and Prevention. CONUS manual for evaluating insecticide resistance in mosquitoes using the CDC bottle bioassay kit. CDC, Atlanta, GA.

McAllister, J. C., M. Porcelli, J. M. Medina, M. J. Delorey, C. R. Connelly, M. S. Godsey, N. A. Panella, N. Dzuris, K. A. Boegler, J. L. Kenney, et al. 2020. Mosquito control activities during local transmission of Zika virus, Miami-Dade county, Florida, USA, 2016. Emerg. Infect. Dis. 26: 872–880.

NACCHO. 2017. National Association of County and City Health Officials. Mosquito control capabilities in the US. NACCHO, Washington DC.

Owusu, H. F., N. Chitnis, and P. Müller. 2017. Insecticide susceptibility of Anopheles mosquitoes changes in response to variations in the larval environment. Sci. Rep. 7: 3667.

Paris, M., G. Tetreau, F. Laurent, M. Lelu, L. Despres, and J. P. David. 2011. Persistence of *Bacillus thuringiensis israelensis* (Bti) in the environment induces resistance to multiple Bti toxins in mosquitoes. Pest Manag. Sci. 67: 122–128.

Parker, C., D. Ramirez, C. Thomas, and C. R. Connelly. 2020. Baseline susceptibility status of Florida populations of Aedes aegypti (Diptera: Culicidae) and Aedes albopictus. J. Med. Entomol. 57: 1550–1559.

Paul, A., L. C. Harrington, L. Zhang, and J. G. Scott. 2005. Insecticide resistance in *Culex pipiens* from New York. J. Am. Mosq. Control Assoc. 21: 305–309.

Raghavendra, K., P. S. Velamuri, V. Verma, N. Elamathi, T. K. Barik, R. M. Bhatt, and A. P. Dash. 2017. Temporo-spatial distribution of insecticide-resistance in Indian malaria vectors in the last quarter-century: need for regular resistance monitoring and management. J. Vector Borne Dis. 54: 111–130.

Ranson, H., and N. Lissenden. 2016. Insecticide resistance in African Anopheles Mosquitoes: a worsening situation that needs urgent action to maintain Malaria control. Trends Parasitol. 32: 187–196.

Ranson, H., R. N'guessan, J. Lines, N. Moiroux, Z. Nkuni, and V. Corbel. 2011. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol. 27: 91–98.

Rao, D. R., T. R. Mani, R. Rajendran, A. S. Joseph, A. Gajanana, and R. Reuben. 1995. Development of a high level of resistance to *Bacillus sphaericus* in a field population of *Culex quinquefasciatus* from Kochi, India. J. Am. Mosq. Control Assoc. 11: 1–5.

Reid, M. C., and F. E. McKenzie. 2016. The contribution of agricultural insecticide use to increasing insecticide resistance in African malaria vectors. Malar. J. 15: 107.

Richards, S. L., J. G. Balanay, M. Fields, and K. Vandock. 2017. Baseline insecticide susceptibility screening against six active ingredients for *Culex* and *Aedes* mosquitoes in the United States. J. Med. Entomol. 54: 682–695.

Rodcharoen, J., and M. S. Mulla. 1996. Cross-resistance to *Bacillus sphaericus* strains in *Culex quinquefasciatus*. J. Am. Mosq. Control Assoc. 12: 247–250.

Rosenberg, R., N. P. Lindsey, M. Fischer, C. J. Gregory, A. F. Hinckley, P. S. Mead, G. Paz-Bailey, S. H. Waterman, N. A. Drexler, G. J. Kersh, et al. 2018. Vital signs: trends in reported vectorborne disease cases—United States and Territories, 2004–2016. MMWR. 67: 496–501.

Sawby, R., M. J. Klowden, and R. D. Sjogren. 1992. Sublethal effects of larval methoprene exposure on adult mosquito longevity. J. Am. Mosq. Control Assoc. 8: 290–292.

Shragai, T., E. Mader, and L. C. Harrington. 2018. Egg Identification Guide for *Aedes albopictus* in the Northeast, USA. Cornell University, Ithaca, NY.

Su, T., J. Thieme, C. Ocegueda, M. Ball, and M. L. Cheng. 2018. Resistance to *Lysinibacillus sphaericus* and other commonly used pesticides in *Culex pipiens* (Diptera: Culicidae) from Chico, California. J. Med. Entomol. 55: 423–428.

Su, T., J. Thieme, G. S. White, T. Lura, N. Mayerle, A. Faraji, M. L. Cheng, and M. Q. Brown. 2019. High resistance to *Bacillus sphaericus* and susceptibility to other common pesticides in *Culex pipiens* (Diptera: Culicidae) from Salt Lake City, UT. J. Med. Entomol. 56: 506–513.

Vasquez, M. I., M. Violaris, A. Hadjivassilis, and M. C. Wirth. 2009. Susceptibility of *Culex pipiens* (Diptera: Culicidae) field populations in Cyprus to conventional organic insecticides, *Bacillus thuringiensis* subsp. *israelensis*, and methoprene. J. Med. Entomol. 46: 881–887.

WHO. 2016a. Monitoring and managing insecticide resistance in Aedes mosquito populations: interim guidance for entomologists. Global Malaria Program, Geneva, Switzerland.

WHO. 2016b. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Global Malaria Program, Geneva, Switzerland.

WHO. 2017. WHO specifications and evaluations for public health pesticides: chlorfenapyr. Global Malaria Program, Geneva, Switzerland.

Xu, T., D. Zhong, L. Tang, X. Chang, F. Fu, G. Yan, and B. Zheng. 2014. *Anopheles sinensis* mosquito insecticide resistance: comparison of three mosquito sample collection and preparation methods and mosquito age in resistance measurements. Parasit. Vectors. 7: 54.