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Research Article

High Resistance to *Bacillus sphaericus* and Susceptibility to Other Common Pesticides in *Culex pipiens* (Diptera: Culicidae) from Salt Lake City, UT

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Abstract

Biorational mosquito larvicides based on microbial organisms and insect growth regulators (IGRs) have played a vital role in integrated mosquito control, particularly since the invasion of West Nile virus to the United States in 1999. Products that are formulated with technical powder of the bacterium, *Bacillus sphaericus* Neide (recently *Lysinibacillus sphaericus* Meyer and Neide), are among the ones that have been extensively applied to combat *Culex* and other mosquito species. Due to the simplicity of the binary toxins, resistance to this pesticide in laboratory and field populations of *Culex pipiens* L. complex has occurred globally since 1994. A *Cx. pipiens* population with a high level of resistance to *B. sphaericus* (VectoLex WDG) was identified in Salt Lake City, UT, in September 2016. The resistance ratios in this population were 20,780.0- and 23,926.9-fold at LC_{50} and LC_{90}, respectively, when compared with a susceptible population of a laboratory reference colony of the same species. This *B. sphaericus*-resistant population remained mostly susceptible to other commonly used pesticides to control arthropods of public health and urban significance, including ones based on microbial organisms (*Bacillus thuringiensis* subsp. *israelensis*, spinosad, spinetoram, abamectin), IGRs (pyriproxyfen, methoprene, diflubenzuron, novaluron), organophosphate (temephos), neonicotinoid (imidacloprid), phenylpyrazole (fipronil), oxadiazine (indoxacarb), and pyrethroid (permethrin). Results are discussed according to the modes of action of the pesticides tested, and suggestions are made to manage *B. sphaericus*-resistant mosquito populations.

Key words: *Bacillus sphaericus*, *Culex pipiens*, Diptera, Culicidae, resistance

Upon economic development and demographic growth, burdens of vectors and vector-borne diseases have been on the rise globally. Mosquito control by means of environmentally sustainable methods is facing severe challenges for numerous reasons, such as range expansion of vector species, newly emerging and increased distribution of mosquito-borne pathogens, and insecticide resistance development. This last obstacle is due in part to the lack of new active ingredients and formulations. Among the few available mosquito larvicides with microbial origins, *Bacillus sphaericus* Neide, recently *Lysinibacillus sphaericus* Meyer and Neide (Ahmed et al. 2007) plays a unique role in combating peridomestic *Culex* spp. Among many strains that have been isolated and identified from natural soil environments, strains 2362, 1597, 2297, C3-41, and IAB-59 possess high larvicidal activity mainly against *Culex* spp. and some other species in the genera of *Anopheles*, *Culiseta*, *Psorophora*, and *Aedes* (Su 2016a). Since the 1960s, strain 2362 has been the interest of research and development worldwide, with a strong emphasis in the United States. Active strains produce parasporal inclusions during sporulation, containing crystal binary toxins A and B (Bin A 42 kDa and Bin B 51 kDa) that play the predominant role in larvicidal toxicity. Some strains also produce noncrystal mosquitocidal toxins (Mtx) such as Mtx 1, 2, and 3 during the vegetative stage. The Mtx contribute to the larvicidal activity less than the binary toxins but are helpful in minimization of resistance development to the binary toxins. Products based on *B. sphaericus* are highly effective against target larval stages in habitats with high organic matter. These formulations offer desired initial and residual efficacy and possess high safety to nontarget species and the environment. The recycling of *B. sphaericus* in mosquito larval carcasses under laboratory and field conditions (Correa and Yousten 1995) further highlights the uniqueness of this naturally occurring microbial organism. Product development and application have been intensified because of increased mosquito control need since invasion and spread of West Nile virus (WNV) in North America.

Resistance to *B. sphaericus* was reported for the first time in 1994, where a high level of resistance occurred in field populations of *Culex pipiens* L. in southern France in response to repeated treatments (Sinègre et al. 1994). Numerous reports on various levels of resistance in laboratory and field populations ensued from different countries (Su 2016b). The first case of resistance to *B. sphaericus* in *Cx. pipiens* in North America was reported recently, where a population from an urban environment in Northern California exhibited high level of resistance to *B. sphaericus* and low susceptibility to other commonly used pesticides against agricultural and urban pests (Su et al. 2018b). In this article, we report another case of high-level resistance to *B. sphaericus* in an urban population of *Cx. pipiens* that was collected from Salt Lake City, UT, in 2016, after a control failure by VectoLex WSP (*B. sphaericus*) was realized in response to application according to the product label. The susceptibility of this population to other commonly used pesticides belonging to different Insecticide Resistance Action Committee (IRAC) groups was also evaluated, and results are discussed according to their modes of action.

Materials and Methods

Mosquitoes

The second instars of *Culex* spp. in the amount of approximately 3,000–4,000 were collected from two to three typical curbside catch basins (1.5′ W × 3′ L × 3′ D) at each of the following four locations in Salt Lake City and neighboring cities, UT on 21 September 2016, by personnel of Salt Lake City Mosquito Abatement District (2020 N. Redwood Rd., Salt Lake City, UT 84116) and South Salt Lake Valley Mosquito Abatement District (7308 Airport Rd., West Jordan, UT 84084): 1400 W. Talisman Dr., Salt Lake City, UT; 9827 Gainlock Cir. and 11010 S. Redwood Dr., South Jordan, UT; and 13200 S. 800 E, Draper, UT (Fig. 1). Collected specimens were shipped to the West Valley Mosquito and Vector Control District (1295 E. Locust St., Ontario, CA 91761) on the same day of collection by overnight shipping. Field-collected larvae were reared to early third instars when species identification was confirmed to be *Cx. pipiens*. Bioassays on *B. sphaericus* were conducted concurrently on all four field collections, along with a laboratory reference colony of the same species from the San Mateo County Mosquito and Vector Control District (1351 Rollins Rd., Burlingame, CA 94010), which was originated from a field collection in Santa Clara, CA, in 2010. There was no pesticide exposure since colonization. Remaining larvae from the field collection that showed a high level of resistance to *B. sphaericus* in the bioassay were colonized for future bioassays on other commonly used pesticides at F_6 when adequate number of larvae was spared for assays when maintaining the colony at the same time. During these six generations, colony was maintained by approximately 10,000–15,000 larvae at each generation to maintain the genetic representation and resistance stability in a confined population (Amorim et al. 2010).

Pesticides

The product that was used to evaluate susceptibility of field populations to *B. sphaericus* was VectoLex WDG (650 ITU/mg; Table 1). The other pesticides used in the susceptibility profile evaluation were also off the shelf products for the relevance to field operations, including five with microbial origins (*Bacillus thuringiensis* subsp. *israelensis*, combination of *B. t. israelensis* and *B. sphaericus*, spinosad, spinetoram, and abamectin); four insect growth regulators (IGRs; methoprene, pyriproxyfen, diflubenzuron, and novaluron); and one from each of organophosphate (temephos), neonicotinoid (imidacloprid), phenylpyrazoles (fipronil), oxadiazine (indoxacarb), and pyrethroid (permethrin). Detailed information for the products used in bioassays is listed in Table 1. These pesticides are either commonly used mosquito larvicides or ones to control urban and household pests with consideration of unintentional exposure and interactive resistance development in mosquitoes.

Bioassays

Cup Bioassay on *B. sphaericus*

VectoLex WDG was suspended in tap water at 200 mg/20 ml to generate a 1% stock suspension; 10× serial dilutions were made for appropriate treatments. Bioassays were conducted as described in previous publication (Su et al. 2018b). Four to five concentrations resulting in approximately 5–95% mortality plus untreated controls were used in bioassays, with three replicates at each concentration. Twenty-five larvae were placed in 100-ml tap water in a 120-ml disposable Styrofoam cup in each replicate. Three drops of 10% rabbit chow pellet suspension were added to each cup as larval food. Bioassays were conducted at 25 ± 1°C. Early third instars were used, and larval mortality was recorded at 48 h post-treatment. Moribund larvae that showed minimum locomotion in response to disturbance (tapping on the bioassay cups) were also considered dead. Concentration–response data were analyzed using POLO-PC to calculate lethal concentrations (LC_{50} and LC_{90}), their 95% confidence intervals (CIs), and slopes of the concentration-response lines. Data heterogeneity (*H* value) was generated by χ^2/df. The bioassay results were accepted when *H* values were ≤2.5 where good probit model fitness was indicated (LeOra Software 1987). Bioassays were also conducted concurrently on a reference laboratory colony of *Cx. pipiens*.

Cup Bioassays on Other Pesticides

To prepare the bioassay treatments, the emulsifiable concentrate formulations such as Radiant SC, Altosid liquid larvicide, NyGuard IGR, ImidaPro 4SC, and Taurus SC were suspended in tap water by gentle mixing. Small granules as in Advance 375A Ant Bait, Advion RIFA Bait, and VectoBac WDG, or wettable powders as in Dimilin 25W were suspended in tap water by vigorous shaking. Large granular materials of VectoMax CG or pellets of Skeeter Abate were pulverized in a coffee grinder (Hamilton Beach Custom Grind, Southern Pines, NC) at the maximum speed, then suspended in tap water by vortexing for 3 min (Vortex Mixer VX100, Labnet International, Inc., Edison, NJ). For briquets such as Mosquiron 0.12CRD, fine pieces were shaved off using a razor blade and suspended in tap water by vortexing for 5 min (Su et al. 2018b). In bioassays on *B. t. israelensis*, the combination of *B. t. israelensis* and *B. sphaericus*, spinetoram, abamectin, temephos, imidacloprid, fipronil, and indoxacarb, late third instars were used, and larval mortality was recorded at 24 h post-treatment because of fact action of these pesticides. In bioassays using diflubenzuron and novaluron, early third instars were preferred, and results were recorded at 72 h post-treatment in relation to the slow action of chitin synthesis inhibitor. Moribund larvae were recorded as dead. In bioassays of methoprene and pyriproxyfen, late fourth instars were used, and mortality was read when all treated individuals emerged as adults or died prior to emergence, as majority mortality occurred during the transition from late larvae,

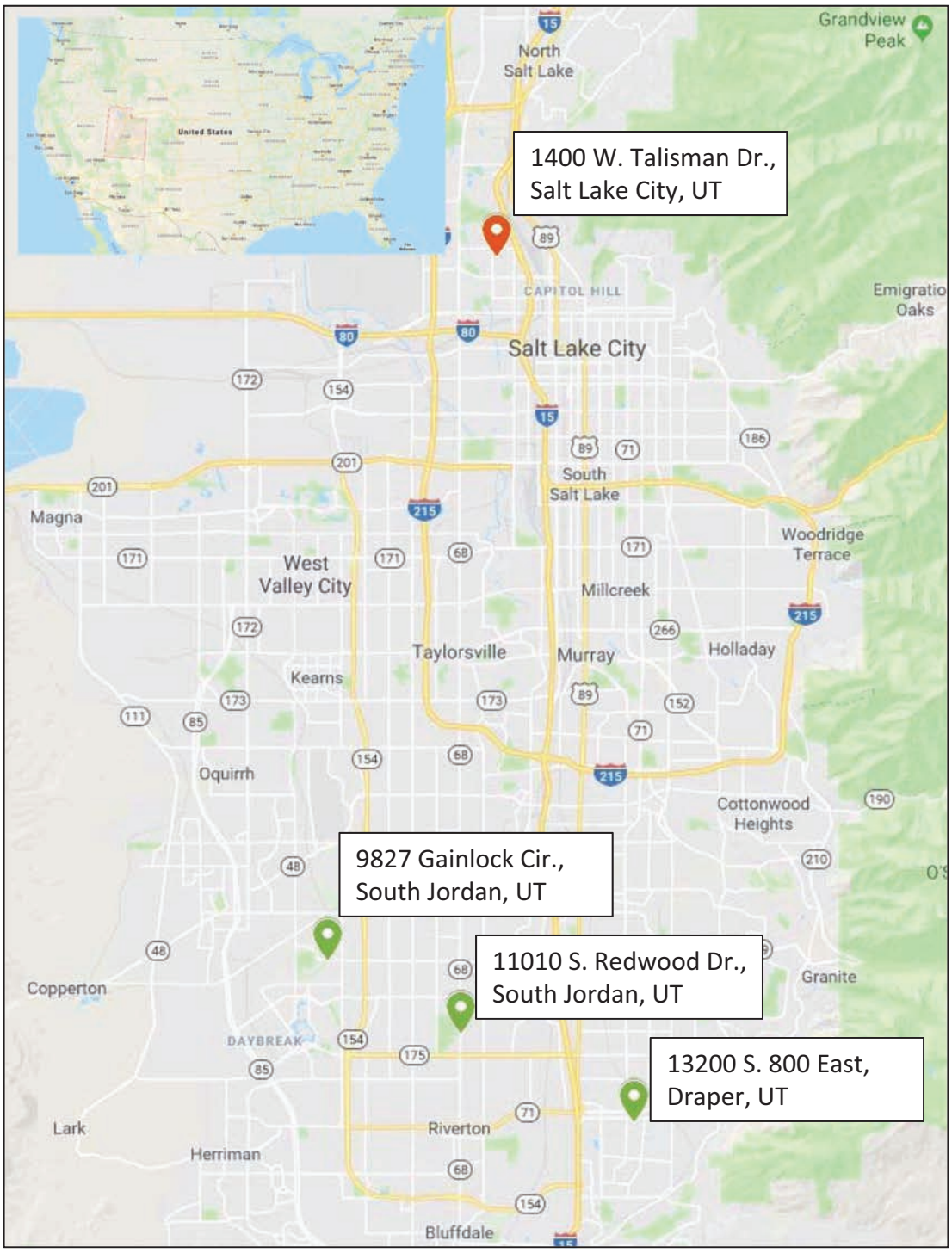

Fig. 1. Sampling sites showing *Bacillus sphaericus*-resistant population in Salt Lake City, UT (red mark), and *B. sphaericus*-susceptible populations in South Jordan and Draper, UT (Green mark).

through pupae to adults. Three drops of 10% rabbit chow pellet suspension were added to each cup as larval food in all bioassays, except those using methoprene and pyriproxyfen where a small piece (approximately 100 mg) of rabbit pellets was added to each bioassay cup to support them until pupation. Concentration–response data were analyzed as previously described. A reference laboratory colony of the same species was assayed concurrently for each pesticide for the calculation of resistance ratio (RR; Su et al. 2018b).

Bottle Bioassay on Adulticide

Bottle bioassays were conducted as described previously (Brogdon and McAllister 1998). As a benchmark for pyrethroid, permethrin was chosen to be assayed against adults. Briefly, technical permethrin (99.9% purity, Chem Service, West Chester, PA) was dissolved in HPLC grade acetone (EMD Millipore, Temecula, CA), and serial dilutions were made to coat the interior of each 250-ml glass bottle (Uline, Pleasant Prairie, WI) at 30 µg/1 ml acetone per bottle. An

Table 1. Information of the pesticides tested against a natural population of *Culex pipiens* L. from Salt Lake City, UT, along with a laboratory reference colony

Category	Products	Active ingredients	Concentration (%)	Lot no.	Manufacturer
Pesticides of microbial origins	VectoLex WDG	*Lysinibacillus sphaericus*	51.2	188-119-PG	Valent BioSciences Corp., Libertyville, IL
	VectoBac WDG	*Bacillus thuringiensis israelensis*	37.4	201-391-PG	Valent BioSciences Corp., Libertyville, IL
	VectoMax CG	*B.t. israelensis* + *L. sphaericus*	4.5 + 2.7	187-575-N8	Valent BioSciences Corp., Libertyville, IL
	Natular G30	Spinosad	2.5		Clarke, St. Charles, IL
	Radiant SC	Spinetoram	11.7	XG14164911	Dow AgroSciences LLC, Indianapolis, IN
	Advance 375A	Abamectin B_1	0.011	81040334	BASF Corp., St. Louis, MO
IGR	Altosid liquid larvicide	Methoprene	5.0	60111357	Wellmark International, Schaumburg, IL
	NyGuard IGR	Pyriproxyfen	10.0	BAB6111	MGK, Minneapolis, MN
	Dimilin 25W	Diflubenzuron	25.0	BA9D30P001	Chemtura Corp., Middlebury, CT
	Mosquiron 0.12CRD	Novaluron	0.12	1012818	Makhteshim Agan North America Inc., Raleigh, NC
Organophosphate	Skeeter Abate	Temephos	5.0	1009280003	Clarke Mosquito Control Products, Inc., Roselle, IL
Neonicotinoid	ImidaPro 4SC	Imidacloprid	40.7	12254PO42	Agrisel USA, Inc., Suwanee, GA
Phenylpyrazole	Taurus SC	Fipronil	9.1	23204	CSI Control Solutions, Inc., Pasadena, TX
Oxadiazine	Advion RIFA bait	Indoxacarb	0.045	SBO45TX	DuPont, Wilmington, DE
Pyrethroid	Permethrin (Technical)	Permethrin (mixture of isomers)	99.9	2601000	Chem Service, West Chester, PA

automatic roller (Fisher Scientific, Fisher Scientific, Hampton, NH) placed in a chemical fume hood (Hemco, Independence, MO) was used to ensure the evenness of the coating. Bottles for untreated controls were coated by acetone only. Bottles were completely dried in the chemical fume hood. Twenty-five 3- to 5-d-old female mosquitoes were aspirated into each bottle. Mortality was read at 5, 10, 15, 30, 45, 60, 90, and 120 min when majority adults died at this coating dose, three replicates were made. Mortality referred to individuals that did not show any movements of body or appendages. Moribund mosquitoes that showed occasional limb (legs and wings) movement were also counted as dead. Bioassays were conducted at 25 ± 1°C and relative humidity 50–60%. Time–mortality data were analyzed for calculations of lethal times LT_{50} and LT_{90}, their 95% CI, and other parameters (Throne et al. 1995). Bottle bioassays were also concurrently conducted on a reference laboratory colony of *Cx. pipiens* for calculation of RRs.

RR Calculation

The RRs were calculated by LC $(LT)_{Field}$/LC $(LT)_{Lab}$. The significance at $P < 0.05$ in susceptibility levels and RRs was determined by separated 95% CIs of LC or LT levels between field population and laboratory colony.

Results

High Resistance to *B. sphaericus*

The field collection from 1400 W. Talisman Dr. in Salt Lake City, UT (red mark in Fig. 1) showed significantly lower susceptibility with LC_{50} 103.9 ppm (95% CI = 76.39–126.64 ppm) and LC_{90} 622.1 ppm (95% CI = 455.09–868.55 ppm) than the other three populations from 9827 Gainlock Cir. and 11010 S. Redwood Dr. in South Jordan, UT, 3200 S. 800E in Draper, UT (green marks in Fig. 1) and the laboratory colony ($P < 0.05$) to VectoLex WDG. The differences in LC_{50} and LC_{90} among these three field populations and laboratory colonies were not significant as indicated by the overlapping 95% CI ($P > 0.05$). Compared with the laboratory colony, the population at 1400 W. Talisman Dr. showed 20,780.0- and 23,926-fold resistance to *B. sphaericus*, whereas no resistance was detected in the other three populations that were collected from South Jordan and Draper, UT (Table 2).

Susceptibility to Other Pesticides

Among the other 13 pesticides tested against larvae and one against adults, the *B. sphaericus*-resistant population only showed significantly lower susceptibility to the combination of *B. t. israelensis* and *B. sphaericus* at LC_{50} level and to permethrin at LT_{90} level (Table 3), and the resistance levels when compared with laboratory reference colony were only 2.36-fold to the former and 4.14-fold to the latter. There were no differences in susceptibility to other 11 pesticides tested between this *B. sphaericus*-resistant population and the laboratory colony of the same species (Table 4).

Discussion

Globalization, emerging, re-emerging, or resurgence of vectors and vector-borne diseases demand cost-effective vector control in an environmentally sustainable manner. Products based on *B. sphaericus* such as VectoLex CG, VectoLex WDG, VectoLex WSP, Spheratax SPH 50G, and Spheratax SPH WSP are among the few tools used in mosquito control operations to combat *Culex* and other mosquito species. The advantages of *B. sphaericus* as one of the most

Table 2. Susceptibility (LC with 95% CI) and RRs to *Bacillus sphaericus* Neide (VectoLex WDG) in F_0 of a natural population of *Culex pipiens* L. from Salt Lake City, UT, in comparison with a laboratory reference colony

Locations	LC_{50} (ppm; 95% CI)	LC_{90} (ppm; 95% CI)	Slope	*H* value[a]	RR at LC_{50}	RR at LC_{90}
1400 W. Talisman Dr., Salt Lake City, UT	103.9[a] (76.39–126.64)	622.1[a] (455.09–868.55)	1.65 ± 0.14	0.80	20,780.0[a]	23,926.9[a]
9827 Gainlock Cir., South Jordan, UT	0.008[b] (0.004–0.013)	0.020[b] (0.013–0.106)	3.21 ± 0.36	1.90	1.60[b]	0.77[b]
11010 S. Redwood Dr., South Jordan, UT	0.008[b] (0.005–0.012)	0.025[b] (0.016–0.073)	2.67 ± 0.29	1.20	1.60[b]	0.96[b]
13,200 S. 800E, Draper, UT	0.004[b] (0.001–0.005)	0.010[b] (0.007–0.024)	2.94 ± 1.02	0.19	0.80[b]	0.38[b]
Laboratory colony	0.005[b] (0.003–0.006)	0.026[b] (0.016–0.087)	2.40 ± 0.38	0.56	1.00[b]	1.00[b]

[a]$H = \chi^2/df$.

[b]Significances in LCs and RRs were indicated by separated 95% CI ranges ($P < 0.05$).

important mosquito larvicides include high activity, tolerance to organic matter, spore recycling, as well as safety to nontarget species and the environment. However, mosquito larvae can develop resistance to *B. sphaericus* in response to sublethal exposures under laboratory and field conditions. Resistance levels vary tremendously in relation to *B. sphaericus* strains, mosquito species, and strains. Presumably this variation is due to historic exposure to other naturally occurring strains, treatment regimens, and gene exchange between treated and untreated populations (Su 2016b). Since 1994, *B. sphaericus* resistance has been reported in *Cx. pipiens* complex in France, India, Brazil, China, Thailand, Tunisia (Su 2016b), and most recently the United States (Su et al. 2018b). The highest level of resistance was revealed in a population of *Culex quinquefasciatus* Say in Nonthaburi Province, Thailand, after five treatments within 4 mo. The RRs at LC_{50}, depending on reference susceptible colonies, were 21,100- to 28,100-fold against VecoLex WDG or greater than 125,000- to 200,000-fold against technical-grade material with 2,000 ITU/mg (Su and Mulla 2004). In North America where the *B. sphaericus* products have been mostly developed and applied against WNV vectors during the past decade, high-level *B. sphaericus* resistance was reported for the first time in a *Cx. pipiens* population collected from Chico, CA, where the RRs were 530.7-fold at LC_{50} and 9,048.5-fold at LC_{90} when compared with laboratory reference colony of the same species. This population from Chico, CA, also showed various levels of resistance or tolerance to abamectin, pyriproxyfen, permethrin, and indoxacarb (Su et al. 2018b). In current studies, the population at 1400 W. Talisman Dr. in Salt Lake City, UT, showed an RR of 20,780.0-fold at LC_{50} and 23,926-fold at LC_{90} to *B. sphaericus*. These levels of resistance were much higher and had much closer RRs at LC_{50} and LC_{90} when compared with what was determined earlier in northern California. These close RRs at LC_{50} and LC_{90} indicate the *B. sphaericus*-resistant population from Salt Lake City was more homogenous compared with the one from northern California (Su et al. 2018b). It is believed that continuous applications of *B. sphaericus* product in the same area for approximately 12 yr was attributable to the high levels of resistance reported here in this article. It is generally thought that recessive genetic factor(s) may govern the *B. sphaericus* resistance in mosquitoes. The mechanism of resistance involves failure of binary toxins to bind to their receptor in the brush-border membrane of the midgut epithelial cells because of compromised integrity of the receptor and/or the glycosylphosphatidylinositol anchor. Other mechanism such as behavioral avoidance in feeding may also play some roles (Nielsen-LeRoux et al. 1995, Darboux et al. 2002, Su 2016b). Limited data have been published (Wirth et al. 2001, Su et al. 2001) regarding *B. sphaericus* susceptibility in natural populations of *Cx. pipiens* in North America prior to Su et al. (2018b) and the current studies. Differences in *B. sphaericus* susceptibility among the populations across California are limited (Wirth et al. 2001), and reduced susceptibility has been observed in larval populations found in dairy wastewater lagoons after continuous applications of *B. sphaericus* product for only one season (Su et al. 2001). Under the field conditions, if expected efficacy according to product label and previous field experience was not achieved post-treatment (control failure) when there were no other factors attributable to reduced efficacy existed such as improper product handling (Su et al. 2018a), substantial habitat dilution, extremely high organic levels, inappropriate application, and sampling, etc., the possibility of reduced susceptibility even resistance in the target population should be considered and bioassay is recommended against the product of interest. Mostly, a simple rotation or mixing (if technically feasible) of pesticides with different modes of action by IRAC can be a practice to minimize resistance development, even though rotation of *B. t. israelensis* and *B. sphaericus* with intention to prevent *B. sphaericus* resistance could be an exception where resistance to *B. sphaericus* developed faster in rotation than in mixture of both (Zahiri and Mulla 2003).

Among the other pesticides tested, the *B. sphaericus*-resistant population was as susceptible as a laboratory reference colony to *B. t. israelensis*, another microbial mosquito larvicide with the same mode of action (IRAC Group 11—microbial disruptors of insect midgut membranes and derived toxins). This microbial larvicide appears to possess an intrinsic mechanism of prevention of resistance development, as resistance to intact natural *B. t. israelensis* is rare regardless of mosquitoes' resistance status to other pesticides (Su 2016b). Reduced susceptibility or tolerance to the combination of *B. t. israelensis* and *B. sphaericus* was detected at LC_{50} level (RR = 2.36) in this *B. sphaericus*-resistant population, which indicated that high-level resistance to *B. sphaericus* may have compromised its susceptibility to the mixture of *B. t. israelensis* and *B. sphaericus*. The known synergism of *B. t. israelensis* to *B. sphaericus* (Su and Mulla 2004, Wirth et al. 2004, Sreshty et al. 2011) did not overcome the resistance to *B. sphaericus* completely. For other pesticides against larval stages tested, widely ranging from ones with microbial and IGR origins to newly developed neonicotinoid, phenylpyrazole, oxadiazine, as well as a conventional organophosphate, no noticeable shift in susceptibility was observed. Therefore, these pesticides can be candidates, depending on registration status, to combat *B. sphaericus*-resistant mosquitoes. These pesticides, with exception of temephos, are all categorized as reduced risk pesticides by the U.S. Environment Protection Agency (U.S. EPA), and some of them have already been registered for larval mosquito control in the United States, such as spinosad (IRAC Group 5—nicotinic acetylcholine receptor allosteric modulator), methoprene (IRAC Group 7A—juvenile hormone analog), and novaluron (IRAC Group 15—chitin synthesis inhibitor). Others such as diflubenzuron (IRAC Group 15—inhibitor of chitin biosynthesis) and pyriproxyfen (IRAC Group 7—juvenile hormone mimic) do have promising potential for application in mosquito control operations. The former

Table 3. Susceptibility (LC or LT with 95% CI) to other commonly used pesticides in F_6 of a natural population of *Culex pipiens* L. from Salt Lake City, UT, in comparison with a laboratory reference colony

	Laboratory colony				Field collection			
Pesticides tested	LC_{50} (ppm) or LT_{50} (min)[a] (95% CI)	LC_{90} (ppm) or LT_{90} (min)[a] (95% CI)	Slope	*H* value[b]	LC_{50} (ppm) or LT_{50} (min)[a] (95% CI)	LC_{90} (ppm) or LT_{90} (min)[a] (95% CI)	Slope	*H* value[b]
Bacillus thuringiensis israelensis (VectoBac WDG, 37.4%)	0.002 (0.001 to 0.004)	0.013 (0.006 to 0.019)	1.55 ± 0.46	0.33	0.002 (0.001 to 0.004)	0.0013 (0.006 to 0.020)	1.43 ± 0.41	0.86
B. t. israelensis + *Lysinibacillus sphaericus* (VectoMax FG, 4.5% + 2.7%)	0.114[c] (0.067 to 0.154)	0.591 (0.451 to 0.931)	1.79 ± 0.31	0.23	0.269[c] (0.239 to 0.301)	0.597 (0.505 to 0.756)	3.70 ± 0.39	0.36
Spinosad (Natular G30, 2.5% spinosyns A & D)	9.75×10^{-3} (5.70×10^{-3} to 1.87×10^{-2})	2.65×10^{-2} (1.52 to 9.95×10^{-2})	2.95 ± 0.30	2.38	9.20×10^{-3} (6.08×10^{-3} to 1.46×10^{-2})	1.90×10^{-2} (1.30×10^{-2} to 0.70×10^{-1})	4.11 ± 0.38	2.36
Spinetoram (Radiant, 11.7% spinosyns J & L)	1.29×10^{-3} (1.05 to 1.40×10^{-3})	3.98×10^{-3} (3.16 to 5.15×10^{-3})	2.55 ± 0.27	0.15	1.52×10^{-3} (8.70×10^{-4} to 2.20×10^{-3})	3.24×10^{-3} (2.25 to 7.73×10^{-3})	3.88 ± 0.37	1.01
Abamectin (Advance 375A, 0.011%)	0.024 (0.018 to 0.036)	0.107 (0.061 to 0.287)	1.97 ± 0.30	0.13	0.014 (0.011 to 0.020)	0.109 (0.056 to 0.383)	1.43 ± 0.24	0.46
Pyripro × yfen (NyGuard, 10%)	5.90×10^{-5} (4.50 to 7.40×10^{-5})	3.12×10^{-4} (2.35 to 4.61×10^{-4})	1.78 ± 0.19	0.40	8.40×10^{-5} (3.00×10^{-5} to 1.67×10^{-4})	3.13×10^{-4} (1.85×10^{-4} to 3.38×10^{-3})	1.98 ± 0.20	2.12
Methoprene (Altosid LL, 5%)	2.08×10^{-3} (1.58 to 2.76×10^{-3})	1.62×10^{-2} (1.04 to 3.06×10^{-2})	1.44 ± 0.15	0.42	1.92×10^{-3} (1.39 to 2.64×10^{-3})	2.31×10^{-2} (1.37 to 4.98×10^{-2})	1.19 ± 0.13	0.18
Diflubenzuron (Dimilin 25 WP, 25%)	6.72×10^{-4} (2.85×10^{-4} to 1.10×10^{-3})	1.02×10^{-2} (6.55×10^{-3} to 2.09×10^{-2})	1.09 ± 0.18	0.64	4.72×10^{-4} (1.75 to 8.04×10^{-3})	5.21×10^{-3} (3.57 to 9.27×10^{-3})	1.23 ± 0.22	0.10
Novaluron (Mosquiron 0.12CRD, 0.12%)	1.08×10^{-3} (8.58×10^{-4} to 1.42×10^{-3})	7.43×10^{-3} (4.40×10^{-3} to 1.82×10^{-2})	1.53 ± 0.22	0.85	8.12×10^{-4} (6.98 to 9.50×10^{-4})	2.62×10^{-3} (2.04 to 3.73×10^{-3})	2.52 ± 0.26	0.72
Temephos (Skeeter Abate, 5%)	3.75×10^{-3} (2.17 to 4.64×10^{-3})	6.50×10^{-3} (4.79 to 8.92×10^{-3})	3.12 ± 0.40	0.92	7.32×10^{-4} (4.47 to 9.81×10^{-4})	2.83×10^{-3} (2.26 to 3.89×10^{-3})	2.18 ± 0.35	0.74
Imidacloprid (ImadPro 4SC, 40.7%)	0.075 (0.052 to 0.108)	0.242 (0.158 to 0.498)	2.52 ± 0.22	1.71	0.075 (0.040 to 0.101)	0.265 (0.142 to 0.466)	3.51 ± 0.21	1.54
Fipronil (Taurus SC, 9.1%)	1.02×10^{-3} (8.59×10^{-4} to 1.2×10^{-3})	3.44×10^{-3} (2.74 to 4.70×10^{-3})	2.44 ± 0.25	0.47	3.03×10^{-4} (1.49 to 4.07×10^{-4})	8.36×10^{-4} (6.89×10^{-4} to 1.14×10^{-3})	2.91 ± 0.67	0.29
Indoxacarb (Advion RIFA bait, 0.045%)	0.170 (0.43 to 0.202)	0.519 (0.413 to 0.702)	2.65 ± 0.25	0.50	0.223 (0.108 to 0.472)	0.671 (0.357 to 1.282)	2.68 ± 0.29	2.15
Permethrin	3.70 (2.06 to 5.47)	46.88[b] (35.68 to 67.78)	1.16 ± 0.14	0.84	8.30 (5.26 to 11.39)	193.87[b] (123.81 to 387.75)	0.94 ± 0.11	0.21

[a]Bottle bioassay.

[b]$H = \chi^2/df$.

[c]Significances in LCs or LTs were indicated by separated 95% CI ranges ($P < 0.05$).

Table 4. RRs to other commonly used pesticides at LC_{50} and LC_{90} or LT_{50} and LT_{90} in F_6 of a natural population of *Culex pipiens* L. from Salt Lake City, UT, in comparison with a laboratory reference colony

Pesticides tested	At LC_{50} or LT_{50}[a]	At LC_{90} or LT_{90}[a]
Bacillus thuringiensis israelensis	1.00	1.00
B. t. israelensis + *Lysinibacillus sphaericus*	2.36[b]	1.01
Spinosad	0.94	0.70
Spinetoram	1.18	0.81
Abamectin	0.58	1.02
Pyriproxyfen	1.42	1.20
Methoprene	0.92	1.42
Diflubenzuron	0.70	0.51
Novaluron	0.75	0.35
Temephos	0.20	0.47
Imidacloprid	1.00	1.10
Fipronil	0.30	0.24
Indoxacarb	1.31	1.29
Permethrin	2.24	4.14[b]

[a]Bottle bioassay.

[b]Significances in RR were indicated by separated 95% CI of the LC or LT ($P < 0.05$).

used to have U.S. EPA label (Dimilin 25W) to control Chironomid midges, a closely related nematoceran group to mosquitoes, whereas the latter was formulated and labeled (NyGuard IGR) to control a wide variety of flying and crawling arthropods of urban, household, and public health importance, including mosquitoes. The remaining pesticides such as imidacloprid (IRAC Group 4—nAChR agonist), fipronil (IRAC Group 2B—GABA-gated chloride channel antagonist), and indoxacarb (IRAC Group 22—voltage-dependent sodium channel blocker) do not currently have product labels for mosquito control; however, they are commonly used to manage urban and household pests because of their U.S. EPA-reduced risk recognition. Last, this *B. sphaericus*-resistant population did show some tolerance to permethrin (IRAC Group 3A—sodium channel modulator) as an adulticide; however, this tolerance is well expected when one considers the extensive use of pyrethroids in the urban environment to mitigate various arthropod pests including mosquitoes (Zhu et al. 2016). Mosquitoes can be exposed at larval and/or adult stages to pyrethroids of urban uses intentionally or unintentionally, which leads to complicated resistance evolution. Considering the similar mode of action in all pyrethroids, the detected tolerance to permethrin can be indicative to the existence of resistance to other pyrethroids that have been applied in urban environment.

While facing the spread of mosquitoes and mosquito-borne diseases, biorational pesticides based on microbial organisms and IGRs are some of the few available tools we can rely on. Resistance development due to lack of rotation of different modes of action and improper use of products has historically dampened the value of the insecticides and made mosquito control more challenging and complicated. The most effective way of resistance management is risk assessment based on mode of action, strategic monitoring of susceptibility, and implementation of mitigation measures. Among the historic cases of *B. sphaericus* resistance worldwide, the relationship of treatment regimens (rates and durations) and level of resistance seems unpredictable. The safe approach that can be recommended is to measure the *B. sphaericus* susceptibility of the target populations and compare it with that of a susceptible population, before, during, and after continuous treatment. The test material can be technical powder or water-dispersible granules with high potency, which ideally contains the same *B. sphaericus* strain as that in the products intended for field application, even though the susceptibility to various *B. sphaericus* strains can be cross-referenced. Insecticide resistance management should be an important component of all integrated mosquito control programs. Given the increasing global burdens posed by mosquitoes and the pathogens that they vector, in addition to a limited arsenal of available insecticide classes for mosquito control, we highly urge the implementation of an insecticide management program within public health and vector control organizations.

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