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OPERATIONAL NOTE

A CASE REPORT ON PRODUCT ROTATION TO MANAGE SEVERE *LYSINIBACILLUS SPHAERICUS* RESISTANCE IN *CULEX PIPIENS* FROM SALT LAKE CITY, UTAH

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ABSTRACT. The Salt Lake City Mosquito Abatement District (SLCMAD) detected a 20,000-fold resistance to *Lysinibacillus sphaericus* (*Lsph*) in *Culex pipiens* occurring in catch basins of Salt Lake City during 2016. In response, SLCMAD suspended use of *Lsph* and rotated use of spinosyn and s-methoprene products for the next three years. At the end of the third year, *Lsph* was evaluated again and efficacy similar to susceptible colony strains. During the second year of *Lsph* use, technicians observed lack of control of larvae at some urban sites. Bioassays performed during 2021 showed recurrence of some resistance to *Lsph* to varying degrees across SLCMAD urban areas. The rapidity with which resistant phenotypes reemerged clarifies that SLCMAD cannot in the near future rely on repeated use of *Lsph*, even after suspending use for three years and using within-season product rotations. Prior reports in other research groups have found long-term selection to *Lsph*, as is the case at SLCMAD, to not regress in spite of halting use of the products. However, our findings offer some optimism that regression may be relatively quick. More operational review is needed, and future work should characterize resistance alleles in field populations. Collectively, there is a lack of concrete data supporting the prevailing assumptions from adjacent industries that were adopted into mosquito abatement. We provide this short note as additional guidance for mosquito and vector control districts weighing options to remediate *Lsph* resistance.

KEY WORDS Catch basin, insecticide resistance monitoring, larvicide, susceptibility, urban

In 2016, the Salt Lake City Mosquito Abatement District (SLCMAD) detected relatively focalized, but severe, resistance in local *Culex pipiens* L. arising from catch basins only within the SLCMAD service area after long-term reliance on *Lysinibacillus sphaericus* (Myer and Neide) (*Lsph*) for control of mosquito larvae (Su et al. 2019). The product was used in catch basins of Salt Lake City for over 12 years with no rotation of active ingredients. Up to 3 individual applications of *Lsph* were conducted in approximately 17,000 catch basins annually. The product was not rotated because *Cx. pipiens*, in addition to catch basins, also thrives in a variety of other larval habitats within urban/suburban environments. Thus, SLCMAD had speculated that the genetic introgression from these habitats, which include artificial containers in private residences that are inaccessible and receive no larval treatments, may be enough to suppress *Lsph* resistance buildup. However, not only was resistance to *Lsph* detected, but the resistance ratios also exceeded 20,000-fold as compared to areas approximately 27 km (17 miles) away within the Salt Lake City metropolitan region (Su et al. 2019). Insecticide product rotations (Hemingway et al. 1997, Yamamura 2021), mosaic treatment patterns (Hemingway et al. 1997), and multimodal mixtures with different active ingredients (Zahiri and Mulla 2003, Sudo et al. 2018) are proposed methods of preventing or remediating insecticide resistance. Laboratory experiments and mathematical models from several other pest management industries have been used to develop these methods (Sudo et al. 2018, Yamamura et al. 2021).

Managing insecticide resistance in the field is difficult because of poor understanding of how various strategies functionally change the observed resistance in mosquitoes and other vectors (Karunaratne et al. 2018, Lucas et al. 2020). It is assumed that the aforementioned resistance management strategies are effective in vector control, but operational reviews to answer if those same strategies result in changes in the field is currently an area with sparse documented research (Dusfour et al. 2019). At present, plans for restoring field efficacy, especially if resistance alleles are still abundant in the population, are not yielding concrete guidance to mosquito abatement districts for managing resistance that is already prevalent (Hemingway et al. 1997, Ping et al. 2001, Ranson et al. 2010, Macoris et al. 2014). For example, product rotation between *Bacillus thuringiensis* var. *israelensis* de Barjac (*Bti*) and *Lsph* has instigated more acute reversal of resistance development to *Lsph*, whereas mixtures resulted in a slower decline in resistance (Zahiri and Mulla 2003). Cross resistance also is typically unidirectional, with an example being that s-methoprene cross resistance is thus far understood to be unidirectional from s-methoprene to *Lsph* (Su et al. 2019, Su et al. 2021). Does this mean that product rotation should obey a specific order of insecticide classes?

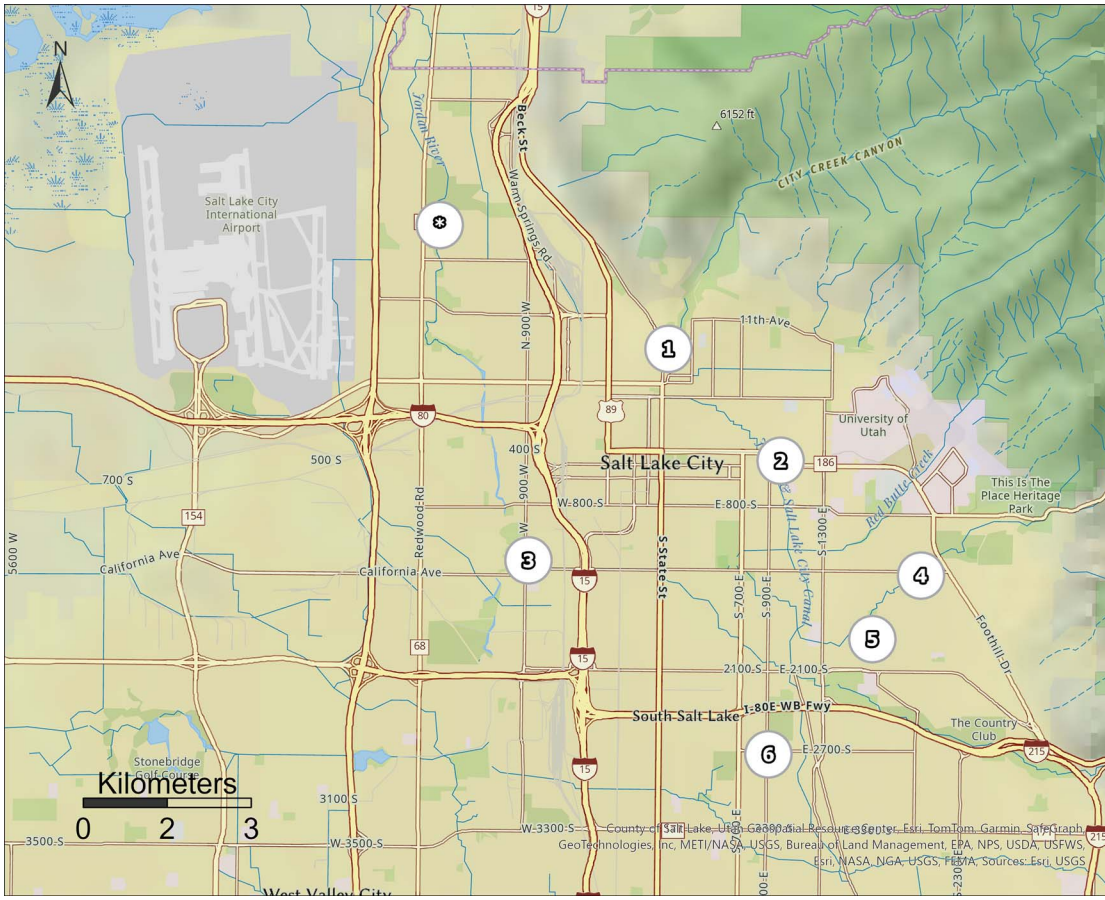
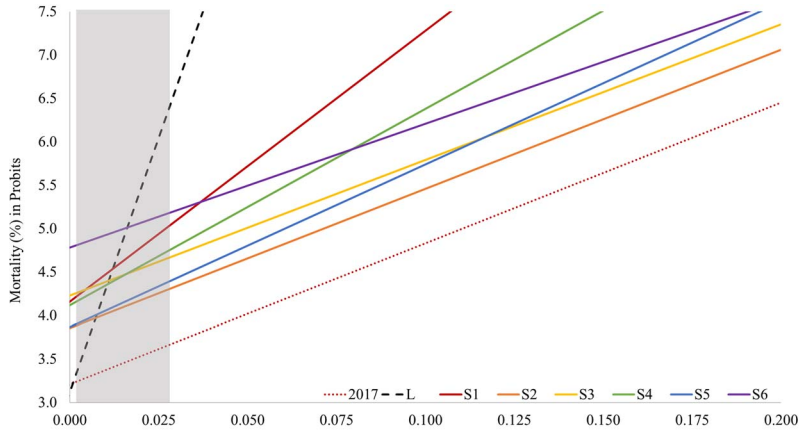


Fig. 1. Sites for collecting *Culex pipiens* L. for use in *Lysinibacillus sphaericus* (Myer and Neide) larval bioassays. The asterisked site is the original reference locality previously diagnosing a critically resistant population of *Cx. pipiens* in the Salt Lake City Mosquito Abatement District.

Other findings show that completely removing selection pressure on *Lsph* did not necessarily reduce the resistance observed in a captive population (Amorim et al. 2007). Is this true in the field as well? Case studies in the USA are generally lacking (Su 2016), with Chico, CA as the only other location reported with severe *Lsph* resistance in the USA (Su et al. 2018). Su et al. (2019) did encounter notable cross resistance to *Bti*, spinosad, spinetoram, abamectin, pyriproxyfen, methoprene, diflubenzuron, novaluron, temephos, imidacloprid, fipronil, indoxacarb, and permethrin when testing the population from SLCMAD. In consequence, SLCMAD implemented a product rotation plan with s-methoprene products exclusively in 2017, spinosyn products exclusively in 2018, and an alternation of s-methoprene and spinosyn products in 2019. Resistance levels were measured in 2017 to see how resistance levels were after a year without *Lsph* selective pressure and for later comparisons. In 2019, efficacy was indistinguishable from colony mosquitoes when using operational rates with *Lsph.*

Consequently, SLCMAD began rotation of s-methoprene products and *Lsph* during 2020. After reports of treatment failures during the 2021 mosquito season resistance was evaluated for and detected again. Results of the assays showed low levels or resistance to *Lsph* in the local *Cx pipiens* populations, which led to the cessation of use of *Lsph* in urban operations since. To contribute an operational review of *Lsph*-resistance mosquitoes, SLCMAD reports here the results of product rotation and testing in a previously diagnosed, highly resistant population of *Cx. pipiens* using the 2017 and 2021 data as a pre/post assessment.

Wild *Cx. pipiens* egg rafts were harvested multiple times across seven sampling areas (Fig. 1) using plastic bins of alfalfa infused water prepared with 1g/liter in tap water and fermented for three to five days. The original diagnostic population with resistance was tested in 2017 (Fig. 1) to establish a reference point. When sampled again in 2019 at the same site (Fig. 1), an 11 lb/acre mid-label rate of VectoLex FG® (650 ITU/mg *Lsph*, Valent Biosciences LLC, Libertyville, IL) returned 100% efficacy in both the



	Slope ± SD	LC_{50} ppm	Lower 95%	Upper 95%	LC_{90} ppm	Lower 95%	Upper 95%	RR_{50}	RR_{90}
2017	0.719 ± 0.13	0.187	0.065	0.538	2.536	0.883	7.285	37.49	97.54
L	4.20 ± 0.35	0.005	0.008	0.018	0.026	0.022	0.050	1.00	1.00
S1	2.10 ± 0.48	0.014	0.009	0.022	0.058	0.036	0.093	2.76	2.21
S2	2.01 ± 0.50	0.063	0.038	0.105	0.276	0.167	0.457	12.67	10.61
S3	1.55 ± 0.65	0.036	0.020	0.065	0.243	0.135	0.438	7.17	9.34
S4	2.56 ± 0.39	0.032	0.021	0.047	0.116	0.077	0.173	6.31	4.45
S5	1.92 ± 0.52	0.041	0.023	0.074	0.192	0.106	0.348	8.23	7.39
S6	1.42 ± 0.70	0.015	0.007	0.030	0.117	0.058	0.236	2.93	4.49

Fig. 2. Probit line graphs with 3rd instar *Culex pipiens* L. mortality from *Lysinibacillus sphaericus* exposures ranging from 0.0025 – 0.1 ppm of VectoLex FG®. Larvae collected from 2017 (dashed line) to establish resistance expression baseline; laboratory susceptible strain (L, solid black line) and field collected mosquitoes from six additional field sites (S1–S6) in 2021. Shaded area is the mortality range up to LC_{90} from the laboratory colony. The table shown at the bottom includes data for the corresponding slopes, $LC_{50/90}$, 95% limits, and resistance ratios.

colony strain and the field strain of mosquitoes. In 2021, an additional six sites were sampled across all areas in which VectoLex products were used operationally during 2020. Field-collected mosquitoes and an established SLCMAD laboratory strain of *Culex quinquefasciatus* Say were reared at consistent environmental conditions of 28 ± 1°C temperature and 70 ± 5% RH. Larvae were fed ad libitum with a 4% alfalfa powder slurry. Both laboratory colony and field-collected larvae were used in bioassays at 2nd – 3rd instar to ensure feeding on treatments.

Treatments were conducted by dissolving VectoLex FG in reverse osmosis (RO) water, then serially diluting in additional RO water until reaching desired test concentrations of 0.0025, 0.0100, 0.0250, 0.0500, and 0.1000 ppm of the formulated product (Su et al. 2019). As a reference point to label rates, the 0.1 ppm concentration was equivalent to 0.906 lb of VectoLex (5–20 lb label rates) per acre assuming a 7.6-cm (3-in) water depth. This dose response range was used in the 2017 and 2021 testing conducted across the SLCMAD service area. Larval bioassays were conducted with Styrofoam cups and 100 ml of RO water (negative control) or 100 ml of treated water at the aforementioned concentrations. Mortality readings were taken 24 h after introducing larvae into the test system. Data were analyzed in PoloPlus (Version 1.0, LeOra Software LLC, Cape Girardeau, MO). Probit-mortality conversions were charted across the *Lsph* concentration gradient by site (Fig. 2).

Prior diagnostics using susceptible portions of the local population provided an LC_{90} value at 0.025 ppm of VectoLex FG (Su et al. 2019). The laboratory reference strain of *Cx. quinqeufasciatus* displayed similar susceptibility, whereas the 2017 benchmark only reached 10% mortality at the same exposure and 40% mortality at 0.1 ppm, with a resistance ratio (RR_{50}) of 37.49 at the LC_{50} (Fig. 2). After product rotation without *Lsph* from 2017 through 2019, we had initially found restored susceptibility when testing mid-label rate of VectoLex FG. In contrast, rotating between s-methoprene and *Lsph* in 2020 resulted in the 2021 field samples still being less susceptible than the laboratory colony (Fig. 2), with site 2 (Fig. 1) being the most resistant of the contemporary samples at an RR_{50} of 12.67. Sites 3, 4, and 5 results had similar slopes with RR_{50} of 2.76, 7.17, 6.31, and 8.23, respectively (Fig. 2), whereas sites 1 and 6 had dissimilar slopes (Fig. 2) but were the most susceptible with RR_{50} of 2.76 and 2.93, respectively (Fig. 2).

Assessments from the original report functionally determined that an application ~1.5 times the maximum label rate still failed to kill SLCMADs resistant *Cx pipiens* (Su et al. 2019). It was previously insinuated that, whenever mosquitoes have undergone long-term pressure from this larvicide, *Lsph* resistance is fixed in spite of avoiding continued selection pressure (Amorim et al. 2007). The population of resistant mosquitoes in Salt Lake City were likely mixing with susceptible populations, such as those to the south of

SLCMAD (Su et al. 2019), but to what extent is unknown. In our follow-up, we can infer that heterogeneity of *Lsph* susceptibility was reestablished by 2019, but three years of product rotation was insufficient to fully restore susceptibility. The split efficacy implies that resistance alleles were still widely distributed with low to no proportion of naïve populations at the time (Karunaratne et al. 2018, Lucas et al. 2020). Nonetheless, the rapidity with which resistant phenotypes reemerged clarifies that SLCMAD cannot in the near future rely on adding *Lsph* to routine larval control operations, even when compensating by rotating products within the season. Especially if there are unclear cross-resistance mechanics amid the product rotation (Su et al. 2021). Despite this, we are optimistic that regression may be relatively quick since the resistance ratios are dramatically less than the original assessments (Su et all. 2019).

To our knowledge, this geography did not demonstrate prior cross-resistance to other common larvicides and adulticides (Su et al. 2019). However, it is possible, though not demonstrated, that the currently known one-way cross-resistance from s-methoprene to *Lsph* (Su et al. 2021) could maintain some level of *Lsph* resistance. In a broader context, it would be worth exploring if there is genetic introgression within these populations or if populations of *Cx. pipiens* found in catch basins are adapting exclusively to this environment. Outside of gene flow arguments, it is difficult to measure allele frequency changes resulting from active insecticide resistance management strategies against mosquitoes (Karunaratne et al. 2018). The genetic basis for resistance has rarely been characterized from field strains with *Lsph* resistance (Su 2016). Both the US-based cases have not been investigated beyond fundamental toxicology (Su 2016, Su et al. 2019, Su et al. 2021). More work needs to be conducted to establish guidelines on tactics like product rotation, mosaics, mixing, and refuge strategies (Sternberg and Thomas 2018). Our case report insinuates that product rotation does help with resistance management in *Cx. pipens*. But overall, there is a need to investigate the broader insecticide resistance management doctrines of mosquito abatement without relying on the assumptions from adjacent industries.

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REFERENCES CITED

Amorim LB, Oliveira CMF, Rios EM, Regis L, Silva-Filha MHNL. 2007. Development of *Culex quinquefasciatus* resistance to *Bacillus sphaericus* strain IAB59 needs long term selection pressure. *Biol Control* 42:155–160. https://doi.org/10.1016/j.biocontrol.2007.04.007

Dusfour I, Vontas J, David JP, Weetman D, Fonseca DM, Corbel V, Raghavendra K, Coulibaly MB, Martins AJ, Kasai S, Chandre F. 2019. Management of insecticide resistance in the major *Aedes* vectors of arboviruses: advances and challenges. *PLoS Negl Trop Dis* 10:e0007615. https://doi.org/10.1371%2Fjournal.pntd.0007615

Hemingway J, Penilla RP, Rodriguez AD, James BM, Edge W, Rogers H, Rodriguez MH. 1997 Resistance management strategies in malaria vector mosquito control. A large-scale field trial in southern Mexico. *Pest Sci* 51:375–382. http://dx.doi.org/10.1002/(SICI)1096-9063(199711)51:3%3C375::AID-PS636%3E3.0.CO;2-K

Karunaratne SHPP, De Silva P, Weeraratne TC, Surendran SN. 2018. Insecticide resistance in mosquitoes: development, mechanisms and monitoring. *Ceylon J Sci* 47:299–309. http://doi.org/10.4038/cjs.v47i4.7547

Lucas KJ, Bales RB, McCoy K, Weldon C. 2020. Oxidase, esterase, and *KDR*-associated pyrethroid resistance in *Culex quinquefasciatus* field collections of Collier County, Florida. *J Am Mosq Control Assoc* 36:22–32. https://doi.org/10.2987/19-6850.1

Macoris MDD, Andrighetti MTM, Wanderley DMV, Ribolla PEM. 2014. Impact of insecticide resistance on the field control of *Aedes aegypti* in the State of Sao Paulo. *Revista da Sociedade Brasileira de Medicina Tropical* 47:573–578. https://doi.org/10.1590/0037-8682-0141-2014

Ping LT, Yatiman R, Gek LPS. 2001. Susceptibility of adult field strains of *Aedes aegypti* and *Aedes albopictus* in Singapore to pirimiphos-methyl and permethrin. *J Am Mosq Control Assoc* 17:144–146.

Ranson H, Burhani J, Lumjuan N, Black WI. 2010. Insecticide resistance in dengue vectors. *TropIKAnet J* 1:1–12.

Sternberg ED, Thomas MB. 2018. Insights from agriculture for the management of insecticide resistance in disease vectors. *Evol Appl*, 11(4), 404–414. https://doi.org/10.1111%2Feva.12501

Su T. 2016. Resistance and its management to microbial and insect growth regulator larvicides in mosquitoes. *In:* Trdan S., ed. *Insecticides resistance*. Rijeka, Croatia: Intech Europe. p 135–154.

Su T, Thieme J, Cummings R, Cheng ML, Brown MQ. 2021. Cross resistance in s-methoprene-resistant *Cilex quinquefasciatus* (Diptera: Culicidae). *J Med Entomol* 58:398–402. https://doi.org/10.1093/jme/tjaa182

Su T, Thieme J, Ocegueda C, Ball M, Cheng ML. 2018. Resistance to *Lysinibacillus sphaericus* and other commonly used pesticides in *Culex pipiens* (Diptera: Culicidae) from Chico, California. *J Med Entomol* 55:423–428. https://doi.org/10.1093/jme/tjx235

Su T, Thieme J, White GS, Lura T, Mayerle N, Faraji A, Cheng ML, Brown MQ. 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. https://doi.org/10.1093/jme/tjy193

Sudo M, Takahashi D, Andow DA, Suzuki Y, Yamanaka T. 2018. Optimal management strategy of insecticide resistance under various insect life histories: heterogenous timing of selection and interpatch dispersal. *Evol Appl* 11:271–283. https://doi.org/10.1111%2Feva.12550

Yamamura K. 2021. Optimal rotation of insecticides to prevent the evolution of resistance in a structured environment. *Pop Ecol* 63:190–203. https://doi.org/10.1002/1438-390X.12090

Zahiri NS, Mulla MS. 2003. Susceptibility profile of *Culex quinquefasciatus* (Diptera: Culicidae) to *Bacillus sphaericus* on selection with rotation and mixture of *B. sphaericus* and *B. thuringiensis israelensis*. *J Med Entomol* 40:672–677. https://doi.org/10.1603/0022-2585-40.5.672