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PROCEEDINGS AND PAPERS

OF THE

Ninety-fourth Annual Conference of the Mosquito and Vector Control Association of California

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President's Remarks – One Health, One Fight: Connecting vector control to global health

Paula Macedo*

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Conference Overview

The 94th Annual Mosquito and Vector Control Association of California (MVCAC) Conference & Exhibitor Showcase took place February 1–3, 2026, in Rancho Mirage, California. This year's theme, "*One Health, One Fight: Connecting Vector Control to Global Health*," highlighted the increasingly interconnected relationship between human, animal, and environmental health, and emphasized the critical role vector control agencies play within the broader public health framework.

It was an honor to serve as MVCAC President-Elect during this conference and to work alongside the MVCAC membership, AMG, the organizing committee, moderators, volunteers, sponsors, exhibitors, and presenters to bring together another successful annual meeting. The conference welcomed attendees from mosquito and vector control agencies, public health departments, academia, research institutions, industry partners, and allied organizations from throughout California and beyond.

Plenary Session: One Health in Action

This year's plenary session set the tone for the conference by exploring the One Health concept from multiple perspectives, linking global collaboration, statewide public health efforts, operational vector control programs, and personal experience into a unified narrative. The plenary session opened with Dr. Cheryl Stroud, Executive Director of the One Health Commission, who delivered a compelling and inspiring presentation on the importance of implementing One Health thinking, decision-making, and collaboration at all levels of society. Drawing from her experiences in veterinary medicine, academia, research, industry, and nonprofit leadership, Dr. Stroud emphasized that the complex public health challenges of today and tomorrow cannot be solved within isolated disciplines. Her presentation highlighted the urgent need for stronger collaboration among public health agencies, veterinarians, environmental scientists, physicians, researchers, educators, and policymakers. She also discussed the work of the One Health Commission in connecting organizations and professionals around the world, promoting education and collaboration, and supporting initiatives such as One Health Day. Her message reinforced that One Health is

fundamentally a "team sport," requiring communication, partnership, and shared responsibility to protect both public and environmental health.

Dr. Kim Conway, One Health Lead for the California Department of Public Health, followed by connecting those broader global concepts directly to California's public health infrastructure and statewide initiatives. Her presentation highlighted how California agencies are increasingly working across traditional boundaries to improve coordination among human, animal, and environmental health sectors. Drawing from her background in veterinary medicine, epidemiology, and public health leadership, Dr. Conway discussed the importance of building systems that support collaboration before emergencies occur. She emphasized the role of preparedness, surveillance, communication, and interagency partnerships in responding to emerging public health threats. Her presentation also underscored the value of local vector control agencies as critical public health partners within California's growing One Health framework.

Dr. Anne Kjemtrup of the California Department of Public Health's Vector-Borne Disease Section then connected the One Health framework directly to the operational realities of vector-borne disease surveillance and response. Her presentation highlighted the extensive work being conducted throughout California to monitor and investigate diseases such as Lyme disease, hantavirus, plague, babesiosis, anaplasmosis, rickettsial diseases, and other vector-borne threats. Drawing upon her experience as a veterinarian, epidemiologist, and research scientist, Dr. Kjemtrup emphasized the importance of integrated surveillance systems involving wildlife monitoring, small mammal trapping, laboratory diagnostics, vector surveillance, environmental investigations, and public outreach. She also discussed the importance of communication and public education in vector-borne disease prevention, noting how even simple educational tools and visuals can significantly increase public awareness and engagement. Her presentation demonstrated how One Health principles are already embedded within modern vector-borne disease surveillance and response activities throughout California.

The final plenary presentation by West Nile virus survivor Jack Raney brought a deeply personal and emotional perspective to the conference. As a longtime supporter of

mosquito and vector control programs, Mr. Raney shared his personal experience with West Nile virus and the profound impacts the disease had on his life and family. His presentation served as a powerful reminder that behind every surveillance report, mosquito pool, control operation, and public outreach campaign are real people and real communities affected by vector-borne diseases. His remarks highlighted the importance of prevention, public awareness, and sustained investment in vector control programs. For many attendees, his presentation reinforced the direct connection between the technical and operational work performed by vector control agencies and the real-world public health outcomes experienced by the communities they serve.

Scientific Program and Conference Activities

The conference featured an extensive and diverse scientific and operational program, including 106 oral and 22 poster presentations, reflecting the depth of expertise and innovation within the vector control community. Sessions addressed administrative challenges, invasive mosquito species, arbovirus surveillance, vector ecology, pesticide resistance, public outreach, environmental compliance, legislation and regulation, emerging technologies, and operational innovation. Presentations also highlighted the increasing use of tools such as drones, sterile insect techniques (SIT), enhanced surveillance technologies, and data-driven operational strategies.

Additional conference activities included a well-attended Trustee Session that provided valuable information and discussion opportunities for district trustees, as well as an outstanding UAS Workshop that highlighted the expanding role of unmanned aircraft systems in vector control operations. The workshop generated significant interest among attendees as districts throughout California continue exploring drone technology for surveillance and pesticide application activities.

Challenges and Opportunities in Vector Control

The conference theme reflected the reality that vector control agencies operate at the intersection of environmental stewardship, public health protection, and community engagement. California continues to face expanding invasive mosquito populations, increasing pressure from climate variability and extreme weather events, local transmission of imported pathogens such as dengue virus, evolving regulatory requirements, and growing public expectations regarding transparency and communication. At the same time, agencies continue to innovate and adapt through collaboration, research, and operational advancement.

Presentations and discussions throughout the conference reinforced the importance of preparedness, flexibility,

communication, and innovation as districts continue responding to both longstanding and emerging vector-borne disease threats.

The Importance of Collaboration Through MVCAC

One of MVCAC's greatest strengths remains its collaborative structure. California's mosquito and vector control districts vary greatly in size, geography, staffing, and operational capacity, yet the association provides a framework through which agencies can share knowledge, resources, and expertise. Through MVCAC committees, regional collaboration, advocacy efforts, and annual meetings such as this conference, districts strengthen their ability to respond collectively to emerging vector-borne disease threats and operational challenges.

The conference also emphasized the importance of public outreach, communication, and advocacy. In an increasingly complex information environment, vector control agencies must continue building trust with their communities while educating policymakers and the public about the value of organized vector control programs.

Acknowledgments and Appreciation

Special recognition and thanks are extended to the conference speakers, moderators, poster presenters, and session organizers who contributed their time and expertise to this year's program. Appreciation is also extended to the conference sponsors and exhibitors whose continued partnership and support make this event possible each year.

The success of the conference would not have been possible without the dedication and hard work of the organizing committee and conference volunteers. Their professionalism, flexibility, and countless hours of behind-the-scenes work ensured a smooth and successful meeting experience for all attendees.

Finally, sincere thanks are extended to all MVCAC members and conference participants. The work performed daily by vector control professionals throughout California continues to protect communities, reduce disease risk, improve quality of life, and strengthen public health infrastructure statewide.

Conclusion

The 94th MVCAC Annual Conference reinforced that One Health is not simply a concept, but rather a practical framework for collaboration, innovation, and public service. Vector control professionals remain an essential part of that effort, working every day to protect the health of California's communities through science, partnership, and dedication.

What is One Health and WHY does it matter to ‘you’?

Cheryl Stroud*

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The objectives of this talk were to define and help MVCAC attendees understand the full scope of One Health, relate examples of needed cross- and trans- disciplinary collaborations to address many of today’s challenges, and describe how vector control professionals work squarely within the One Health framework. One Health was described as a modern-day resurrection of an ancient understanding of the inextricable interdependence of the environment and all life. Although it was zoonotic diseases that pass between animals and humans that first drove the current One Health discussion, many of today’s most ‘wicked’ challenges, from antimicrobial resistance, avian influenza, climate change, disaster preparedness, food safety and security, to plant and soil health, vector-borne and zoonotic diseases, must be addressed using a One Health approach. Examples of ‘systems’ challenges that have forced us into disciplinary and professional silos were described. True One Health stories were shared demonstrating how and why it is critical that animal, environmental, and human health workers and social scientists develop collaborative working relationships ‘ahead’ of public health emergencies to best ‘prevent’ and ‘respond’ to them. Examples of why such collaborations are urgently needed included true narratives about animals as sentinels of emerging diseases and environmental contaminants, points about humans and animals sharing

common environments, microbiomes and vector-borne disease exposures, explanations of comparative medicine and translational research, the need for One Health planning before and in response to disasters and more. Take-home messages included: 1) No one profession can know ‘everything’, so we must find ways to collaborate. 2) In today’s systems, the needed collaborations will not just magically ‘happen’, they must start with ‘relationships’. 3) In today’s systems, those needed relationships will not just magically happen; we must step out of our comfort zones to create them. 4) Implementation of One Health at local, national, regional or international levels will require both ‘Top Down’ (governments) and ‘Bottom Up’ (community level) understanding, energy and actions. This talk briefly demonstrated how the One Health approach is now being embraced by many organizations and individuals around the world in a global movement. Slides are available at this link. <https://tinyurl.com/Stroud-MVCAC-26>

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OneHealthCommission.org

CDC One Health webpage. <https://www.cdc.gov/one-health/index.html>

One Health is spreading in California

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Abstract

The California Department of Public Health (CDPH) defines One Health as a globally recognized approach that incorporates the interdependency of people, animals, plants, and a shared environment into strategies that achieve optimal health outcomes and well-being for all. The CDPH One Health program works to address complex challenges that exist at the interface of animal, human, and environmental health through outreach and education, research, relationship building, technical assistance, advocacy, and collaboration with local, state, and national partners. Examples of putting One Health into action have included embedding the One Health approach in the State's H5N1 response, operationalizing One Health Clinics to serve people and their pets, and launching regional One Health initiatives that convene multidisciplinary partners across counties to strategize on locally relevant One Health challenges. One Health collaborations will be successful when they are driven by key tenets such as prioritizing relationships, making an effort to understand partner perspectives, celebrating wins and protecting processes that are going well, valuing domestic animals, wildlife, and ecosystems independent from human health, and being inclusive of non-traditional partners. As new threats continue to emerge, the One Health approach is the way forward.

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CDPH One Health Implementation Toolkit for Local Jurisdictions
CDPH One Health in Action - Video

One Health in Vector-Borne Diseases in California: More than a slogan

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Abstract

The One Health concept recognizes the interconnectedness of people, animals, and the environment for the health and benefit of all those domains. The concept is surprisingly intuitive for vector-borne disease prevention and response. It is important to move beyond intuition to implementation to transform how challenges may be addressed fostering cross-sector collaborations, integrating environmental considerations, and reframing complex problems. This presentation uses California examples to show what One Health looks like in action: how understanding Rocky Mountain spotted fever has benefited from a multi-sector approach; how integrated pest management (IPM), often shown as a pyramid, shifts dynamically based on vector biology, human behavior, and environmental context; and how lessons from medicine, such as antimicrobial stewardship, can inform strategies for managing pesticide resistance. There are limitations to the model - including the risk of misinterpreting animal surveillance data to assess human health risk when assumptions underlying the data are not properly addressed, and regulatory rules such as those that restrict pesticide use for public health purposes - must be considered in One Health collaborations. Ultimately, applying a One Health approach offers opportunities to innovate and strengthen vector-borne disease surveillance and response, promoting healthier ecosystems and communities.

William C. Reeves New Investigator Award

The William C. Reeves New Investigator Award is given annually by the Mosquito and Vector Control Association of California in honor of the long and productive scientific career of Dr. William C. Reeves.

The award is presented to the outstanding research paper delivered by a new investigator based on the quality of the study, the manuscript, and the presentation at the MVCAC Annual Conference.

Year	Award Winner	Title of Paper
1988	Vicki L. Kramer	A comparison of mosquito population density, developmental rate and ovipositional preference in wild versus white rice fields in the Central Valley
1989	Truls Jensen	Survivorship and gonotrophic cycle length in <i>Aedes melanimon</i> in the Sacramento Valley of California
1990	Gary N. Fritz	Polytenes, isozymes and hybrids: deciphering genetic variability in <i>Anopheles freeborni</i>
1991	David R. Mercer	Tannic acid concentration mediates <i>Aedes sierrensis</i> development and parasitism by <i>Lambornella clarki</i>
1992	Darold P. Batzer	Recommendations for managing wetlands to concurrently achieve waterfowl habitat enhancement and mosquito control
1993	Jeffery W. Beehler	The effect of organic enrichment and flooding duration on the oviposition behavior of <i>Culex</i> mosquitoes
1994	Merry-Holliday-Hanson	Size-related cost of swarming in <i>Anopheles freeborni</i>
1995	Margaret C. Wirth	Multiple mechanisms cause organophosphate resistance in <i>Culex pipiens</i> from Cyprus
1996	No award	
1997	John Gimmig	Genetic and morphological characterization of the <i>Aedes (Ochlerotatus) dorsalis</i> group
1998	Yvonne Ann Offill	A Comparison of mosquito control by two larvivorous fishes, the stickleback (<i>Gasterosteus aculeatus</i>) and the mosquitofish (<i>Gambusia affinis</i>)
1999	Parker D. Workman	Adult spatial emergence patterns and larval behavior of the "Tule Mosquito," <i>Culex erythrorhox</i>
2000	Jason L. Rasgon	Geographic distribution of <i>Wolbachia</i> in California <i>Culex pipiens</i> complex: infection frequencies in natural populations
2001	Christopher Barker	Geospatial and statistical modeling of mosquito distribution in an emerging focus of La Crosse virus
2002	No award	
2003	Laura Goddard	Extrinsic incubation period of West Nile virus in four California <i>Culex</i> (Diptera: Culicidae) species
2004	No award	
2005	Troy Waite	Improved methods for identifying elevated enzyme activities in pyrethroid-resistant mosquitoes
2006	Lisa J. Reimer	Distribution of resistance genes in mosquitoes: a case study of <i>Anopheles gambiae</i> on Bioko Island
2007	Carrie Nielson	Impact of climate variation and adult mosquito control on the West Nile virus epidemic in Davis, California during 2006
2008	John Marshall	The impact of dissociation on transposon-mediated disease control strategies
2009	Win Surachetpong	MAPK signaling regulation of mosquito innate immunity and the potential for malaria parasite transmission control
2010	Tara C. Thiemann	Evaluating trap bias in bloodmeal identification studies
2011	Sarah S. Wheeler	Host antibodies protect mosquito vectors from West Nile virus infection
2012	Brittany Nelms	Overwintering biology of <i>Culex</i> mosquitoes in the Sacramento Valley, California
2013	Kimberly Nelson	The effect of red imported fire ant (<i>Solenopsis invicta</i> Buren) control on neighborhoods in Orange County, California
2014	Thomas M. Gilbreath, III	Land Use Change and the Microbial Ecology of <i>Anopheles gambiae</i>
2015	Jessica M. Healy	Comparison of the efficiency and cost of West Nile virus surveillance methods in California
2016	Mary Beth Danforth	The impacts of cycling temperature on West Nile virus transmission in California's Central Valley
2017	Nicholas A. Ledesma	Entomological and Socio-behavioral Components of Dog Heartworm (<i>Dirofilaria immitis</i>) Prevalence in Two Florida Communities
2018	Kim Y. Hung	House Fly (<i>Musca domestica</i> L.) Attraction to Insect Honeydew
2019	Matteo Marcantonio	Revising alkali metals as a tool for mark-recapture studies to characterize patterns of mosquito (Diptera: Culicidae) dispersal and oviposition
2020	Adena Why	Semiochemicals associated with the Western mosquitofish, <i>Gambusia affinis</i> , and their effect on the oviposition of <i>Culex tarsalis</i>
2021	Vanessa Hill	Evaluation of residential property types for <i>Aedes aegypti</i> habitats in Placer County, California
2022	Phurchhoki Sherpa	Asian longhorned tick, <i>Haemaphysalis longicornis</i> (Ixodida: Ixodidae), and optimal collection methods for the tick in the Northeast United States
2023	Mark Dery	Effect of Bed Bugs (<i>Hemiptera Cimicidae</i>) aldehydes efficacy of fungal biopesticides
2024	Mario Novelo	Dengue and chikungunya virus loads in the mosquito <i>Aedes aegypti</i> are determined by distinct genetic architectures
2026	Karen Click	Aerial adulticiding reduces infection in West Nile virus vectors

Reeves New Investigator Award 1st Place: Aerial adulticiding reduces infection prevalence in West Nile virus vector populations

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Introduction

Aerial adulticide applications are used during periods of epidemic West Nile virus (WNV) infection risk to rapidly reduce the number of infected adult female mosquitoes. Previous studies have estimated the efficacy of aerial applications by comparing pre- and post-application arboviral infection prevalence in a single treated zone and a nearby untreated comparison zone (Reisen 2010). Results from these studies have been extremely variable, ranging from 22-93% reduction in infection prevalence, likely due to external factors such as weather and season impacting comparisons and short pre- and post-treatment trapping periods limiting available data (Reisen et al. 1984, Nielsen et al. 2007, Elnaïem et al. 2008, Lothrop et al. 2008, Macedo et al. 2010). A recent study from California used 12 years of aerial application and trapping data from the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) to make the first unified estimate of the long-run average magnitude and duration of aerial treatment effects on abundance, finding short-term reductions followed by population rebounds (Holcomb et al. 2021). To extend this previous work, we used 19 years of records from SYMVCD and Placer Mosquito and Vector Control District (PMVCD) to estimate the impacts of aerial application on WNV infection prevalence in *Cx. tarsalis* and *Cx. pipiens*.

Methods

A multiple-baselines interrupted time-series design was used to assess the average effect of aerial adulticide application on WNV minimum infection rate (MIR) in *Cx. spp.* populations (Hawkins et al. 2007, Bernal et al. 2017, Centers for Disease Control 2024). SYMVCD and PMVCD provided records of 1,899 aerial treatment events, including spatial polygons and associated treatment dates (SYMVCD: 2006-2024, PMVCD: 2015-2024). Pooled testing data from mosquitoes captured by CO₂ traps was accessed via the CalSurv Gateway (VectorSurv Development Team 2025) with agency permission. Pools larger than 60 mosquitoes or marked “pending” or “inconclusive” were not included in the analysis, and the remaining pools were

aggregated by trap location and collection date to calculate MIR (n=45,320 pools). Aerial treatment polygons were overlaid using ArcGIS Pro version 3.1.0 (Esri 2023) to identify areas of unique time series of treatments. Observations of WNV MIR from testing data were linked to sequences if they fell within the spatial polygon and were collected within 4 weeks before or after the first treatment. Only sequences which had at least 3 time points trapped and tested for WNV pre- and post-treatment for either *Cx. tarsalis* or *Cx. pipiens* were included for analysis. Included sequences were aggregated for each species and aligned using the initial treatment date. Segmented regression then was used to fit an observed post-treatment trend, an immediate treatment effect, and a counterfactual no-treatment scenario trend extrapolated from the pre-treatment trend and covariates accounting for seasonal and long-term patterns in MIR. Attributes of application events, including spatial coverage (within 5-km buffer of trap location), number of treatments, and seasonal timing (early season vs late season), were assessed via interaction terms in the segmented regression model.

Results and Discussion

After aerial application spatial polygons were overlaid, there were 1,495 polygons of unique sequences of aerial treatments. Of these, a total of 62 sequences met our inclusion criteria for one or both species (*Cx. tarsalis*: n=61, *Cx. pipiens*: n=41); the remaining polygons were excluded because they either 1) contained no traps that yielded mosquitoes that were tested for WNV within 4 weeks pre- or post-treatment (n=1,320) or 2) they had fewer than three time points of WNV MIR observations either pre- or post-treatment (n=113). Significant reductions in infection occurred within several days post-application in *Cx. tarsalis*, with an estimated 1-week mean change in WNV MIR of -36.1% (95% CI -51.8, -15.8) in *Cx. tarsalis* and -10.0 (95% CI -41.9, +38.7) in *Cx. pipiens*. At four weeks post-application, both species displayed significant suppression of WNV MIR compared to the counterfactual no-application scenario, with 4-week mean changes of -75.7%

(95% CI –86.7, –56.3) in *Cx. tarsalis* and –67.6 (95% CI –88.1, –10.8) in *Cx. pipiens*. Both spatial coverage and seasonal timing of aerial application significantly impacted the effectiveness of aerial treatments. These findings agreed with previous studies, which predominantly found reductions in WNV MIR following aerial adulticide application. The interrupted time-series methods used in this study extended prior findings by demonstrating the long-run average reduction using a large dataset of treatment and surveillance records, controlling for confounding, and estimating lagged effects on MIR (Nielsen et al. 2007, Elnaiem et al. 2008, Lothrop et al. 2008, Macedo et al. 2010). We believe this methodology will be valuable for assessing vector control efforts in other contexts in future studies.

Conclusions

Aerial applications of adulticides were shown to result in long-term reductions in WNV MIR in both *Cx. tarsalis* and *Cx. pipiens*. This suppression lasted longer than previous estimates of the impact on abundance, indicating that the impacts on WNV MIR persist beyond population rebounds. Future studies are needed to evaluate the effects of smaller-area adult mosquito control using truck-mounted or backpacks, and to consider the impacts of aerial adulticide applications on human risk of human WNV disease.

Acknowledgements

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Reeves New Investigator Award 2nd Place: Size-mediated activity and reproduction impacts of irradiation for sterile insect technique in *Aedes aegypti* (Diptera: Culicidae)

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Introduction

The expanding ranges of vectors and pathogens (Yi et al. 2014, Ryan et al. 2019) and the challenges of continued management (Zhang et al. 2024) are forming into an increasingly urgent call for new tools, methods, and analyses to bolster integrated vector management practices. The yellow fever mosquito *Aedes aegypti* (Linnaeus) is at the forefront of this struggle (Abbasi 2025) globally, and after invading California during the past decade has risen to prominence across much of the state (Metzger et al. 2017). To mitigate the rise of insecticide resistance and limit ecological harm, species-specific and self-limiting tools such as the sterile insect technique (SIT, Thomé et al. 2010) have been adapted to local *Aedes* populations, validated and deployed via several public health agencies (Birhanie et al. 2025). Although districts vary in their capacity to redirect resources for maintenance and deployment of this technique, all are limited in the ability to conduct auxiliary research into factors that may be important for successful SIT programs. The relationships between radiation dose, rearing conditions, and mating success have been evaluated by previous work focusing on cohort scale responses to select doses that maximize sterility while minimizing the negative impact on function (Chen et al. 2023, Birhanie et al. 2024). However, these approaches lack individual level information and within-cohort variations in potentially important factors such as size, activity, and mating potential. To better characterize the impacts of radiation on *Ae. aegypti* our study had two objectives: 1. Examine the interaction between radiation dosage and male mating success, and 2: Use individual level female data to quantify male competitiveness and determine if female size inhibited reproduction.

Methods

A field derived strain of *Ae. aegypti* was obtained from West Valley Mosquito and Vector Control District (WVMVCD) and maintained under laboratory conditions adequate to not inhibit larval growth and adult emergence.

Pupae from this line were sorted to separate adult males and females before mating. Within 48 h of emergence, male adults were transported to WMVCD and subjected to X-ray radiation from a Rad Source RS 1800-Q machine. Exposure time based dosages used were 0 Gy for controls, 42 Gy “low” dose, and 55 Gy “high” dose. After 24 h rest, 25–32 males per treatment were placed in individual 25 mm vials (1 male per vial) installed in a locomotor activity monitor (LAM25, Trikinetics Inc). The monitor counts the times that a laser field in the center of each vial is crossed, thereby measuring activity. Vials were sealed with a 10% sucrose sugar feeder with continuous data collected for four weeks. A separate set of 30 males were placed in mating cages with 30 females from the same cohort, with the males treated as follows: 30-Control, 30–42 Gy, 30–55 Gy, 15-Control + 15–42 Gy, and 15-Control + 15–55 Gy. After 1 week of cohabitation and blood feeding, females were transferred to individual vials with filter paper and wet cotton for oviposition. Eggs were counted then hatched in the vials one month later, with larval counts occurring 2 days after. Males from the LAM25 and females from oviposition vials had their wings removed and measured to relate size to other metrics.

Results

Overall the response in the males to irradiation resulted in lower survivorship (Fig. 1A) and lower total activity (Fig. 1B) over time, but there was no significant difference between the two different irradiation doses. Separating daily activity monitoring data over time revealed that peak dawn and dusk activity were reduced in the first few days post-irradiation, but in the second week onwards all 3 treatments showed similar activity patterns (Fig. 2). There were no significant changes in diel patterns or circadian rhythm overall. Male size did not significantly interact with treatment for activity results, but there was a significant interaction with treatment on survival with smaller males having higher mortality in the high radiation dose compared to the low. Non-competitive mating results show similar overall sterility patterns as

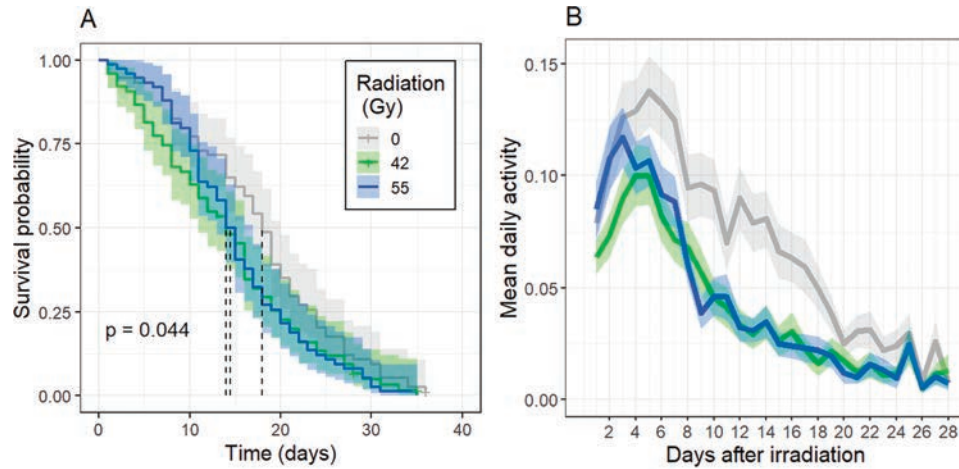


Figure 1.—Summaries of survival and locomotor activity data. **A.** Cohort survival probability plotted as a function of time after irradiation on the x-axis with 95% confidence interval as ribbons for each. Colors denote radiation dosage treatment and vertical dashed lines show median survival times for each group. Model p -value refers to overall effect of dosage when taking size into account, though size on its own was not significant ($p = 0.056$). **B.** The mean proportion of the day classified as ‘active’, averaged across individuals in each treatment with ribbons depicting standard error of the mean. Activity was lower in irradiated males and all activity decreased over time, largely due to cohort mortality, although days was not a significant factor in linear mixed effects models.

previous literature, but individual female fertility revealed heterogeneous male sterility contributions to hatch rate (Fig. 3A) where many males were fully sterilized while others were not. With a 1:1 competition with unirradiated males, eggs showed a similar mean hatch for the two doses. Females with 55 Gy males exhibited a binary response of high hatch or extremely low, whereas with 42 Gy there was a broader range of hatch rates. The mean hatch rates were

equalized by the number of “impaired” matings being higher in the 42 Gy competition scenario. Female size correlated fecundity, but there was no relationship between size and hatch rate across treatments (Fig. 3B), suggesting size did not influence male mating success based on irradiation. These results allowed for the improvement of models estimating overall male performance and life tables for females based on release scenarios.

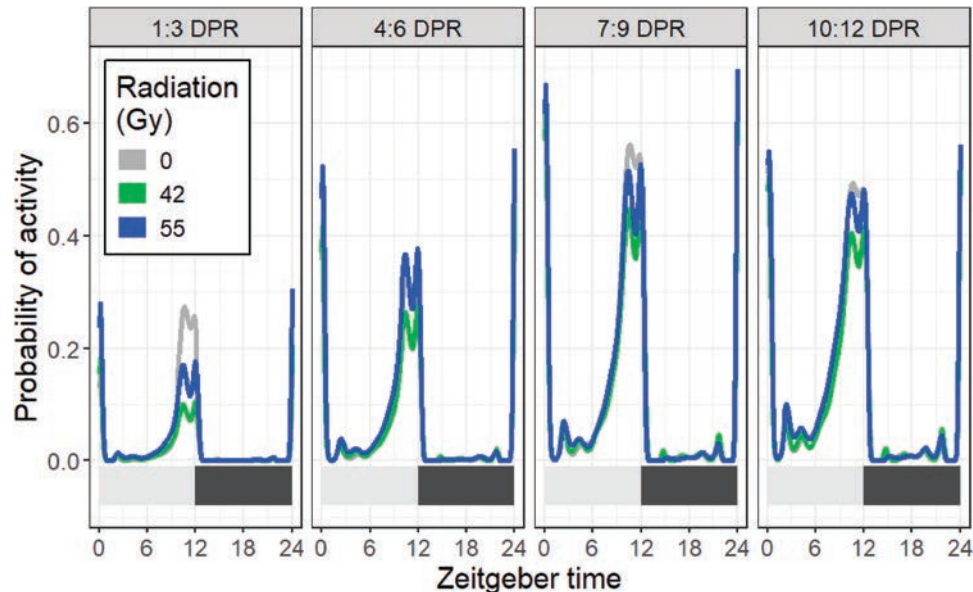


Figure 2.—Diel activity patterns of irradiated male mosquitoes, faceted by three-day post radiation (DPR) blocks. Y-axis represents probability of a single mosquito being active during that 10-minute time block. ZT time is aligned to insectary 12:12 light cycle with 0 corresponding to 8:00 AM. Smooth functions based on treatment interacting with ZT did not produce significant changes in circadian rhythm, so GAM differences are largely associated with changes in dawn and dusk peak amplitudes. Irradiated males in the first time block had a dramatic reduction in peak activity compared to control that tapered across further days, suggesting irradiation stress requires recovery of some kind.

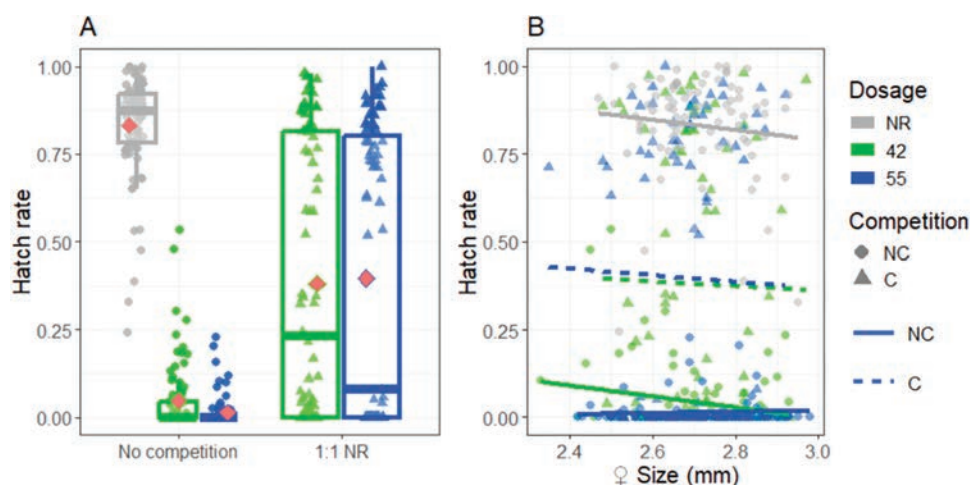


Figure 3.—Patterns in egg hatch for individual females across five mating scenarios. Circles denote non-competitive (NC) mating with one type of male and triangles represent 1:1 competition (C) with radiation treated males and unirradiated control males. **A.** Boxplot of egg hatch rate across the competition scenarios. Diamonds represent the mean hatch rate. Points illustrate heterogeneity in responses across dosages and competition. **B.** The same datapoints as a scatterplot against female size depicting linear trends (solid = NC, dashed = C). No interactions between size and hatch rate were observed, supporting that female size does not mediate discretion or male access between treatments.

Discussion

Overall findings revealed interactions between irradiation and activity and mating competitiveness that are important factors for SIT programs. Although the pooled results resembled previous findings in *Ae. aegypti* and dose-response patterns from other SIT literature (Barry et al. 2003, Helinski et al. 2009), the collection of individual-based data revealed novel patterns and improved our understanding of how sterility must be balanced for deployment. The reduced male activity in the first week post-irradiation presents a novel recovery window where prioritizing the release of younger males may be sub-optimal as reduced early activity curtailed mating potential and survivorship benefits. Lower dosages may have lower sterility, but this may be outweighed by improved survival and ability to mate in field conditions. More data is needed from larger scales to fully model these trade-offs and optimize public health efforts. The lack of sized based female bias in this field derived population is a promising result but needs further examination in semi-field scenarios.

Conclusions

As SIT programs ramp up globally, the methods by which we evaluate their implementation and results require further development. Our research provided an illuminating first foray into these individual-based approaches that will allow for robust evaluation of program parameters and success, which are vital to effective IVM.

Acknowledgements

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Reeves New Investigator Award 3rd Place: Larvicide resistance and cross-resistance in the *Culex pipiens* assemblage

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Introduction

Integrated mosquito management relies heavily on the use of larvicides with different modes of action, and rotating these products is a key strategy for both preventing and remedying resistance (AMCA, 2021; World Health Organization, 2016). Because of this, cross-resistance between larvicide active ingredients with seemingly unrelated modes of action can be detrimental to mosquito control efforts. Recent reports of high resistance to the juvenile hormone analog (JHA) S-methoprene (Lopez et al., 2024), combined with evidence of cross-resistance to *Lysinibacillus sphaericus* in S-methoprene resistant mosquitoes in the laboratory (Su et al., 2020), present a complicating challenge for optimizing mosquito control and larvicide rotation strategies. To better understand cross-resistance relationships in mosquito populations, we used two colonies of *Cx. quinquefasciatus* and *Cx. pipiens*/*quinquefasciatus* with known resistance to S-methoprene and *L. sphaericus*, respectively (Su et al., 2018, 2020). In addition, we determined if resistance status also predisposes mosquitoes for resistance to pyriproxyfen and spinosad larvicides.

Methods

Resistance was maintained in the S-methoprene resistant (MET) and *L. sphaericus* resistant (SPH) colonies by continuing selection pressure generationally throughout the duration of this study using Altosid Liquid Larvicide and Vectox WDG, respectively. Following previously described bioassay protocols (Lopez et al., 2024, Su et al., 2018), we evaluated the susceptibility of the MET colony, the SPH colony, and a laboratory strain of *Cx. pipiens* (IA) (Erickson et al., 2006) to S-methoprene, pyriproxyfen, *L. sphaericus*, and spinosad. Mortality data were adjusted using Abbott's formula (Abbott, 1925), and probit analysis was used to estimate LC₅₀ and LC₉₀ values for each mosquito colony and active ingredient (Finney, 1971; Robertson et al., 2007; Throne et al., 1995; Wheeler et al., 2006). Probit model fits were assessed with Pearson's chi-squared goodness of fit. Resistance ratios (RRs) were calculated to compare the relative resistance or susceptibility

of the resistant colonies using the World Health Organization's classification scale (World Health Organization, 2016).

Results and Discussion

The *Cx. pipiens* (IA) colony was susceptible to JHAs tested including S-methoprene (LC₅₀ of 2.162×10^{-4} ppm and LC₉₀ of 0.103 ppm) and pyriproxyfen (LC₅₀ of 3.401×10^{-5} ppm and LC₉₀ of 0.012 ppm). This colony also showed susceptibility to *L. sphaericus* (LC₅₀ of 7.243×10^{-3} ppm and the LC₉₀ was 0.050 ppm) and spinosad (LC₅₀ of 4.611×10^{-4} ppm and LC₉₀ of 8.203×10^{-3} ppm) (Table 1).

The resistant colonies MET and SPH exhibited high resistance to their respective larvicides. The MET colony had an LC₅₀ of 0.040 ppm (RR of 1.861×10^2) and LC₉₀ of 1.148×10^2 ppm (RR of 1.110×10^3) to S-methoprene (Table 2). The SPH colony had an LC₅₀ of 5.610×10^4 ppm (RR of 7.746×10^6) and an LC₉₀ of 3.370×10^6 ppm (RR of 6.804×10^7) to *L. sphaericus* and was resistant to the point that 100% mortality could not be reached (Table 3).

The MET colony showed high cross-resistance to *L. sphaericus* (LC₅₀ of 0.484 ppm and RR of 66.881; LC₉₀ of 1.071×10^3 ppm and RR of 2.161×10^4) and pyriproxyfen (LC₅₀ of 5.751×10^{-4} ppm and RR of 16.909; LC₉₀ of 0.102 ppm and RR of 8.248) (Table 2). This is in agreement with Su et al. (2020) in which a previous evaluation of the same colony determined cross-resistance to *L. sphaericus* and pyriproxyfen.

Notably, the SPH colony also was resistant to S-methoprene (LC₅₀ of 0.053 ppm and RR of 2.455×10^2 ; LC₉₀ of 14.597 ppm and RR of 1.412×10^2) but susceptible to pyriproxyfen (LC₅₀ 2.770×10^{-6} ppm and RR of 0.081; LC₉₀ of 1.275×10^{-3} ppm and RR of 0.103) (Table 3). The reciprocal cross-resistance relationship between S-methoprene and *L. sphaericus* has not been described previously and was unexpected. These active ingredients are in two distinct groups as defined by the Insect Resistance Action Committee (IRAC). Namely, S-methoprene is in Group 7 (juvenile hormone receptor modulators), and *L. sphaericus* is in Group 11 (microbial disruptors of insect midgut membranes) (Insect Resistance Action Committee, 2025). Resistance mechanisms affecting one group are not

Table 1.—Susceptibility to larvicides in a reference colony of *Culex pipiens*.

Larvicide Tested	Susceptible Colony				
	N	LC ₅₀ (ppm) (95% CI)	LC ₉₀ (ppm) (95% CI)	p-value	Slope ($\pm$ s.e.m.)
S-methoprene	120	2.162×10^{-4} (1.023×10^{-4} – 4.048×10^{-4})	0.103 (0.047–0.282)	4.755×10^{-54}	0.478 ± 0.019
Pyriproxyfen	80	3.401×10^{-5} (1.464×10^{-5} – 7.484×10^{-5})	0.012 (0.003–0.110)	1.142×10^{-74}	0.500 ± 0.024
<i>L. sphæricus</i> (Vectolex® WDG)	92	7.243×10^{-3} (4.930×10^{-3} – 0.011)	0.050 (0.030–0.108)	6.327×10^{-160}	1.534 ± 0.058
Spinosad (Natular® G30)	96	4.611×10^{-4} (3.047×10^{-4} – 7.017×10^{-4})	8.203×10^{-3} (4.551×10^{-3} – 0.018)	3.342×10^{-75}	1.025 ± 0.036

Table 2.—Susceptibility to larvicides in an S-methoprene resistant colony of *Culex quinquefasciatus*.

Larvicide Tested	S-methoprene Resistant Colony				
	N	LC ₅₀ (ppm) (95% CI)	LC ₉₀ (ppm) (95% CI)	RR at LC ₅₀	RR at LC ₉₀
S-methoprene	150	0.040 (0.019–0.088)	1.148×10^2 (24.2–1123.3)	1.861×10^2	1.110×10^3
Pyriproxyfen	125	5.751×10^{-4} (3.88×10^{-4} – 8.68×10^{-4})	0.102 (0.051–0.238)	16.909	8.248
<i>L. sphæricus</i> (Vectolex® WDG)	213	0.484 (0.232–1.112)	1.071×10^3 (1.875×10^2 – 1.625×10^4)	66.881	2.161×10^4
Spinosad (Natular® G30)	90	2.765×10^{-4} (1.949×10^{-4} – 3.917×10^{-4})	3.999×10^{-3} (2.466×10^{-3} – 7.412×10^{-3})	0.600	0.312
				p-value	Slope ($\pm$ s.e.m.)
				1.589×10^{-138}	0.370 ± 0.014
				3.117×10^{-27}	0.569 ± 0.018
				$< 1.000 \times 10^{-100}$	0.383 ± 0.014
				3.719×10^{-47}	1.104 ± 0.039

Table 3.—Susceptibility to larvicides in a *L. sphaericus* resistant colony of *Culex quinquefasciatus*/*pipiens*.

Larvicide Tested	N	<i>L. sphaericus</i> Resistant Colony				
		LC ₅₀ (ppm) (95% CI)	LC ₉₀ (ppm) (95% CI)	RR at LC ₅₀	RR at LC ₉₀	p-value
S-methoprene	138	0.053 (0.035–0.085)	14.597 (5.893–47.261)	2.454 × 10 ²	1.412 × 10 ²	1.166 × 10 ^{–44}
Pyriproxyfen	93	2.770 × 10 ^{–6} (1.181 × 10 ^{–6} –5.417 × 10 ^{–6})	1.275 × 10 ^{–3} (5.616 × 10 ^{–4} –3.882 × 10 ^{–3})	0.081	0.103	7.154 × 10 ^{–34}
<i>L. sphaericus</i> (Vectotex® WDG)	87	5.610 × 10 ⁴ (2.417 × 10 ⁴ –2.196 × 10 ⁵)	3.370 × 10 ⁶ (6.402 × 10 ⁵ –7.159 × 10 ⁷)	7.746 × 10 ⁶	6.804 × 10 ⁷	4.889 × 10 ^{–79}
Spinosad (Natulac® G30)	126	3.148 × 10 ^{–4} (1.482 × 10 ^{–4} –6.929 × 10 ^{–4})	1.969 × 10 ^{–3} (8.615 × 10 ^{–4} –0.010)	0.683	0.004	< 1.000 × 10 ^{–100}
						1.610 ± 0.057
						0.721 ± 0.042
						0.481 ± 0.023
						0.525 ± 0.019

expected to affect another. Interestingly, Lopez et al. (2025) found that S-methoprene resistant *Cx. pipiens* were not cross-resistant to *L. sphaericus*. This shows how variable susceptibility profiles can be among different populations.

Both resistant colonies were susceptible to spinosad. For the MET colony, the LC₅₀ for spinosad was 2.765 × 10^{–4} ppm (RR of 0.600), and the LC₉₀ was 3.999 × 10^{–3} (RR of 0.312) (Table 2). Likewise, the LC₅₀ of the SPH colony was 3.148 × 10^{–4} ppm (RR of 0.683), and the LC₉₀ was 1.969 × 10^{–3} ppm (RR of 0.00648) (Table 3). Although previous assays of the MET colony found low cross-resistance to spinosad (Su et al. 2020), evaluation of field-collected *Cx. pipiens* highly resistant to *L. sphaericus* revealed no cross-resistance to spinosad (Su et al., 2019).

Conclusions

These findings demonstrated that selection of resistance to a single larvicide can produce unexpected cross-resistance to mechanistically different products. Reciprocal cross-resistance between S-methoprene and *L. sphaericus*, and cross-resistance to pyriproxyfen in S-methoprene resistance mosquitoes can reduce the effectiveness of larvicide rotation strategies commonly used in integrated mosquito management. Because of this, proactive resistance surveillance and evidence-based larvicide selection is necessary to sustain effective mosquito control and protect public health.

Acknowledgements

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Exploring the role of geosmin from *Streptomyces exfoliatus* and *Kitasatospora purpeofusca* in attracting gravid *Aedes aegypti* in urban settings

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Introduction

Aedes aegypti is a major public health threat due to its role in transmitting dengue, chikungunya, yellow fever, and Zika viruses, and its close association with humans in urban environments complicates surveillance and control. Current trapping methods rely on a limited number of attractants, highlighting the need for novel, biologically relevant cues (Maia et al., 2024). Geosmin, a microbial volatile associated with larval habitats, has been identified as a potential oviposition attractant (Melo et al., 2020). This study evaluated whether geosmin-producing bacterial isolates (*Streptomyces exfoliatus* and *Kitasatospora purpeofusca*) could enhance the attraction of gravid *Ae. aegypti* under laboratory and field conditions.

Methods

Laboratory oviposition assays were conducted using gravid *Ae. aegypti* females in four-choice oviposition cages, with three treatment cups and one distilled water control per trial. Initial assays evaluated plant-derived extracts (beetroot, carrot, and mushroom) and geosmin across a range of concentrations to identify potential attractants. However, subsequent experiments focused on microbial sources of geosmin, and only bacterial-derived lures were advanced to field trials.

Two geosmin-producing bacterial isolates (LL18 and LL132), obtained from agricultural soils at Cherry Research Farm (Goldsboro, NC) and provided by the Choudoir laboratory at North Carolina State University, were cultured on ISP5 agar and incorporated into lure formulations. Laboratory comparisons evaluated oviposition responses to bacterial cultures grown on ISP5 agar, which were suspended above deionized water-filled oviposition cups to allow exposure to microbial volatiles without direct contact, relative to reference attractants, including gravid infusion (water, grass and yeast fermented for seven days) and deionized water.

Field trials were conducted from July–October 2025 across four urban sites in Fresno County, California:

Braden Court (BC), Clovis Core (CC), Quail Lake (QL), and Sunnyside (SS). A total of 160 trap locations were used, with 20 gravid traps and 20 autocidal gravid ovitraps (AGOs) deployed per site. Traps were operated weekly over a three-month period, and mosquito collections were recorded per trap-night. Experimental traps were baited with bacterial lures (LL18 and LL132), which were rotated between sites every two weeks, while control traps used standard gravid infusion.

Results and Discussion

In laboratory assays, beetroot and beetroot–carrot combinations produced the highest oviposition responses, whereas geosmin alone did not significantly increase egg-laying relative to controls (Fig. 1). Bacteria-based lures consistently resulted in lower egg counts compared to gravid infusion or deionized water, indicating that microbial volatile profiles were not competitive under these conditions.

In field trials, AGO trap collections showed significant site-level differences in female *Ae. aegypti* abundance (Fig. 2). However, no significant differences were observed between bacterial lure treatments and controls. These results indicated that environmental variation among sites had a stronger influence on trap performance than lure type. Contamination of bacterial cultures altered volatile emissions, which appeared to reduce the attraction of *Ae. aegypti* while increasing captures of non-target species, including *Forcipomyia* spp., consistent with responses to microbial volatile organic compounds (Davis et al., 2013).

Conclusions

Although pure geosmin has been shown to stimulate oviposition in *Ae. aegypti* (Melo et al., 2020), bacteria-derived geosmin sources produced inconsistent results in our study. Variability in microbial cultures, particularly due to contamination,

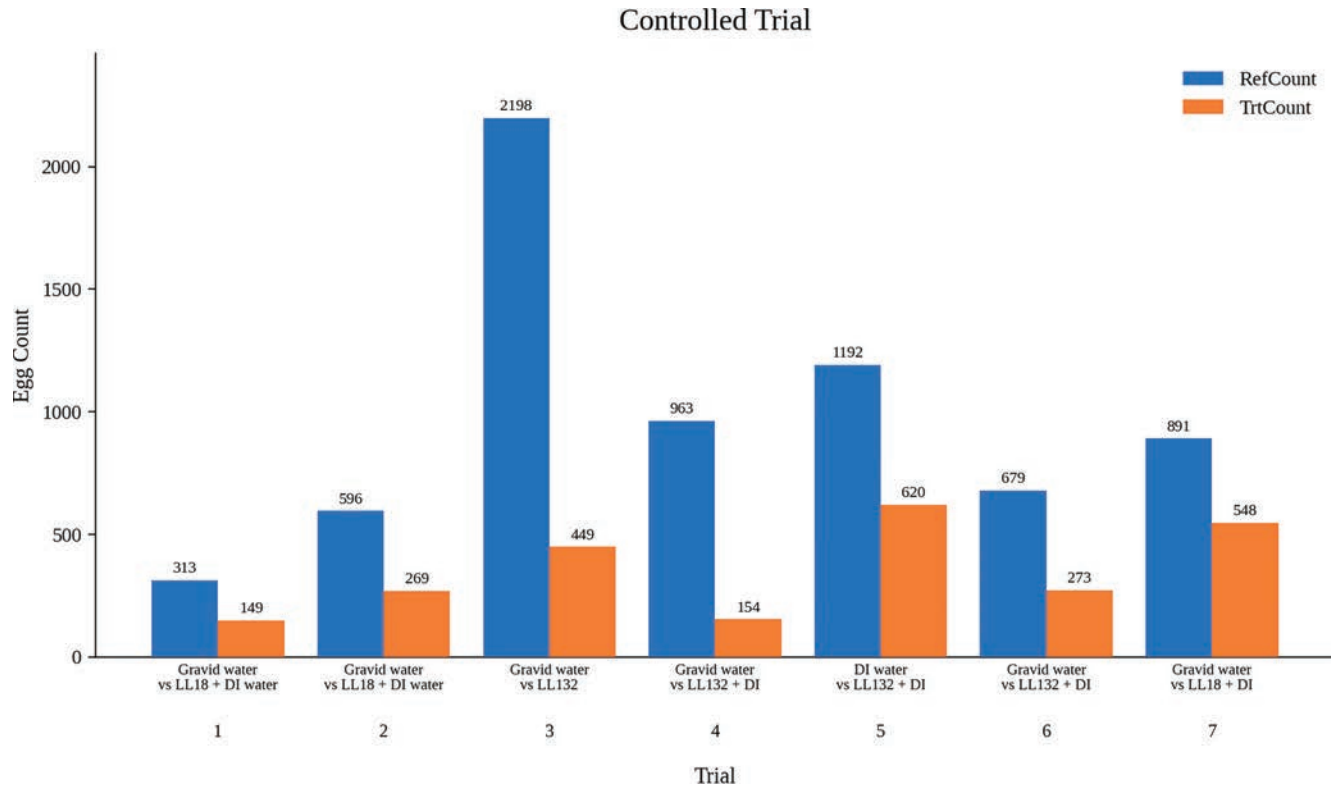


Figure 1.—Laboratory oviposition responses of gravid *Ae. aegypti* to bacteria-based lures compared with reference water. Reference treatments consisted of either gravid infusion (fermented grass and yeast mixture) or deionized water, while treatment cups contained ISP5 agar cultures of isolates LL18 or LL132 (with or without added deionized water). Bacterial cultures were suspended above deionized water-filled oviposition cups to allow volatile exposure without direct contact. Each paired set of bars represents egg counts from a single 24-h trial (Trials 1–7). Across all trials, bacterial treatments resulted in fewer eggs than reference treatments, indicating reduced attractiveness under laboratory conditions.

likely altered volatile profiles and reduced effectiveness. These findings highlight the importance of strict quality control in microbial lure development. Future work should focus on stabilizing microbial

volatile production and integrating these cues with established attractants, such as infusion water, to improve surveillance performance.

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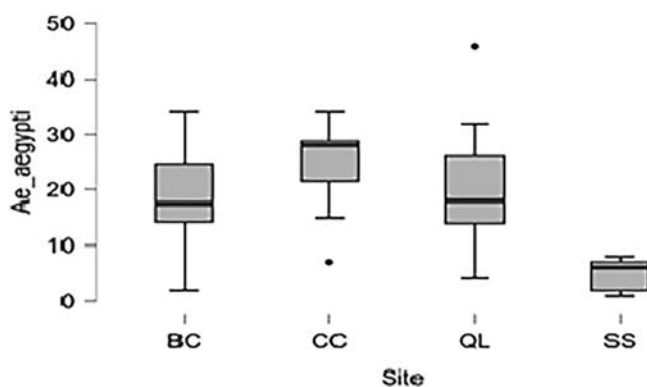


Figure 2.—Female *Ae. aegypti* captured per trap-night in autocidal gravid ovitraps (AGOs) across four urban sites: Braden Court (BC), Clovis Core (CC), Quail Lake (QL), and Sunnyside (SS). Boxplots show the median, interquartile range, and outliers. Significant differences in mosquito abundance were observed among sites, indicating that local environmental conditions and population abundance influenced trap performance.

Oviposition as an indicator of *Wolbachia*-Infected male release efficacy for urban *Aedes aegypti* control

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Abstract

Integrated Vector Management (IVM) is essential for controlling *Aedes aegypti* and reducing vector-borne disease risk in urban environments. The Sacramento–Yolo Mosquito and Vector Control District (SYMVCD) employ Wide Area Larvicide Spraying (WALS) to target immature stages across large residential areas. To complement this larval control strategy, in 2025, SYMVCD initiated a pilot release of *Wolbachia*-infected male *Ae. aegypti* using the Incompatible Insect Technique (IIT) to suppress local populations through induced sterility. This 18-week study focused on evaluating the combined efficacy of these two population suppression strategies in South Natomas, Sacramento, California, using oviposition traps to monitor egg abundance and hatch rate at sixty residential sites that spanned across four treatment zones. Egg abundance followed strong seasonal patterns, with peak oviposition occurring in the late summer to early Fall. In contrast, hatch rate differed across zones with significantly reduced egg viability observed in both the Release Area and Buffer zones relative to the Control and WALS treatment zones. These findings indicate that although oviposition activity is largely driven by environmental factors, hatch rate serves as a more sensitive indicator of IIT efficacy. Ultimately, the results support the integration of reproductive suppression to SYMVCD’s current IVM program to enhance urban *Ae. aegypti* management.

Introduction

Climate change and increasing urbanization have contributed to the expansion of medically important vectors, increasing the global risk of vector-borne disease transmission (Gage et al. 2008; Abbasi 2025). One such species, *Aedes aegypti*, is highly adapted to peridomestic and domestic environments due to behavioral and physiological adaptations such as a preferred anthropophilic feeding preference, use of artificial containers and man-made sources as ovipositing sites, implementation of skip oviposition during gonotrophic cycles, desiccation resistant eggs, and acquiring multiple blood meals prior to ovipositing (Matthews 2019; Facchinelli et al. 2023). These characteristics and patterns have convoluted mosquito control efforts and, in some instances, increased the risk of the transmission of dengue (DENV), Zika, chikungunya, and yellow fever viruses.

These evolved adaptations have not only enabled *Ae. aegypti* to thrive worldwide but have accelerated its establishment and expansion across the state of California (CDPH, 2025). In Southern California, this expansion has been accompanied by 24 locally acquired DENV cases, in recent years, whereas the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) continues to respond to travel-associated cases (Kolimenakis et al. 2021; CDPH 2025). Through rigorous surveillance, SYMVCD has observed and documented ongoing localized expansion and increasing population abundance of *Ae. aegypti* throughout

Sacramento and Yolo counties, particularly in established residential areas such as South Natomas, where populations have continued to exponentially increase, since 2022, despite ongoing implementation of integrated vector management (IVM) efforts.

Current control efforts in this South Natomas area rely on the use of wide area larvicide spraying (WALS) applications. This involves truck-mounted A1 super duty mist sprayers applying Vectobac WDG, which contains the active ingredient *Bacillus thuringiensis israelensis* (*Bti* strain AM65-52), a biorational insecticide designed to target cryptic sources of immature *Ae. aegypti* with no consequential impact on non-target species (Williams et al. 2022). However, because WALS only targets the larval stages, its impact on adult populations is limited. To complement this larval control strategy, SYMVCD initiated a pilot Incompatible Insect Technique (IIT) program in 2025, involving the release of *Wolbachia*-infected male *Ae. aegypti* reared by MosquitoMate, Lexington, KY, USA.

Although *Wolbachia*-infected male mosquito releases and WALS applications represent complementary population suppression strategies, SYMVCD has not previously evaluated their combined effects on *Ae. aegypti* populations at a neighborhood-wide scale. The current study assessed the combined efficacy of these control measures utilizing oviposition traps to record changes in egg abundance and egg viability. By quantifying these metrics, the study provided operational insight into how integrated larval and

reproductive control strategies enhanced urban IVM programs and supported long-term suppression of *Ae. aegypti* populations.

Methods

Study area and experimental design

The study was conducted over the course of 18 weeks in South Natomas, beginning 9 July (epi-week 29) and concluding 20 November 2025 (epi-week 47). The neighborhood was divided into four treatment zones: Control (~42 hectares [ha]), WALS (~165 ha), Buffer (~69 ha), and Release Area (~10 ha), each containing 15 residential sites (60 total sites). Each residential site was selected based on historical data showing adult or larval *Ae. aegypti* detections. Likewise, due to personnel limitations, a randomized generator in Microsoft Excel was used to construct three trapping groups (A, B, and C), which each consisted of 5 sites from each treatment zone (20 sites per group). To maintain a balanced study design, one group was serviced per week, resulting in a three-week rotational trapping schedule throughout the study.

The Control zone received no designated treatments during the study unless residents input service requests for barrier treatments due to day biting mosquito pressure. Conversely, the WALS, Buffer, and Release Area treatment zones all received 9 WALS treatments during the 2025 mosquito season beginning 12 June and concluding 18 September. WALS treatments were conducted for three consecutive weeks and then stopped for one month, prior to re-starting the applications. The Release Area was the only zone that received the IIT treatment. Bi-weekly releases occurred Tuesday and Friday totaling 30,000 *Wolbachia*-infected male *Ae. aegypti* per week. Although the Buffer zone did not receive IIT treatments, it bordered the Release Area to account for potential dispersal-related spillover effects. All zone assignments remained constant for the duration of the study.

Oviposition trap monitoring

Oviposition activity was monitored weekly at 20 residential sites, with two traps deployed per site in shaded front-yard locations. Each trap consisted of a 946 mL black container filled with 500 mL of a fermented gravid water infusion prepared from 10% alfalfa and 3.5 grams Brewer's yeast (Snetselaar et al. 2014; Codeço et al. 2015; James et al. 2022). Infusions were prepared and allowed to ferment for 7 days prior to deployment. Traps were interiorly lined with pre-cut seed germination paper, which was labeled with each site's unique MapVision site identification number. After a 7-day deployment period, traps were retrieved and egg papers were transported to the laboratory in labeled bags for processing and storage.

Egg processing and storage

Ovi-papers were examined under a stereomicroscope, and all *Aedes* eggs were counted. Positive ovi-papers were dried

under laboratory conditions for 24 hours between paper towels and glass plates to standardize drying evenness. This was followed by an additional 13 day drying period, under laboratory conditions in darkness to assist in standardizing embryonation prior to conducting emergence assays (Zheng et al. 2015).

Hatch rate and emergence assays

After the 14-day drying period, ovi-papers were submerged in 1.6L containers, containing 1,400 mL dechlorinated water, 20 mL nutrient broth, and 30 mg Brewer's yeast (Wohl and McMeniman 2023). A small quantity (15 mg) of ground TetraMin was added to stimulate hatching within 24–48 hours of immersion (Benedict et al. 2020). After 48 hours, larvae were pipette-transferred to BioQuip emergence containers containing 400 mL dechlorinated water. Larvae were fed 15 mg of TetraMin daily (excluding weekends). After 7 days, emerged larvae and adults were verified as *Ae. aegypti*, utilizing a morphological key.

Eggs that appeared collapsed, desiccated, or otherwise structurally compromised under microscopic examination were classified as inviable and excluded from hatch rate calculations. While some inviable eggs may have resulted from infertility or environmental stress, viability was assessed based solely on morphological characteristics, and the specific cause of inviability was not determined. Hatch rate was therefore calculated as:

$$\frac{\text{total larvae, pupae, and emerged adults}}{(\text{total \# of eggs collected}) - (\text{\# of inviable eggs})} \times 100\%$$

Data analysis

Statistical analyses were done in R v4.4.2 (R Core Team 2024). Two-way ANOVA and Tukey HSD post hoc tests were used to examine variances in *Ae. aegypti* egg abundance and hatch rate across trapping zones.

Results

In total, 14,134 eggs were collected from the South Natomas neighborhood (Figure 1, 2). Egg abundance (n = 360 egg trap collections) data were natural log-transformed [$\ln(x + 1)$] to meet the assumptions of normality and homoscedasticity required for ANOVA. A two-way ANOVA then was performed on log-transformed egg abundance data to evaluate the effects of treatment zone and epidemiological week on *Ae. aegypti* oviposition activity. Following data transformation, the model showed a significantly strong effect of epi-week on egg abundance ($P < 0.001$), indicative of temporal/seasonal variations impact on oviposition activity (Figure 3). In contrast, treatment zone and zone*epi-week interaction did not significantly influence egg abundance ($P = 0.43$, $P = 0.70$, respectively) (Figure 2).

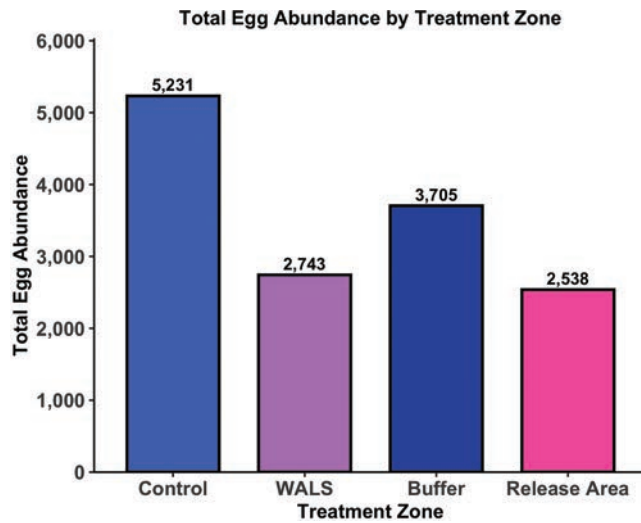


Figure 1.—Total number of *Aedes aegypti* eggs collected by treatment zone over the 18-week study period. Values represent the sum of eggs collected across all ovi-traps within each treatment zone.

For hatch rate data, the two-way ANOVA of untransformed data, revealed both treatment zone ($P < 0.001$) and epi-week ($P < 0.001$) significant effects, whereas the interaction of zone and week was not significant ($P = 0.15$), indicating that weekly patterns in the hatch rate were similar across zones. However, Tukey's HSD post hoc test comparisons revealed several significant differences among zones. These differences included significantly lower hatch rates for homes in the Release Area compared to those in the control ($P < 0.001$) and WALS ($P < 0.001$) zones (Figure 5). Likewise, homes in both the Control ($P = 0.03$) and WALS ($P = 0.03$) zones had significantly higher hatch rates than those in the Buffer zone, indicating potential dispersal of *Wolbachia*-male *Ae.*

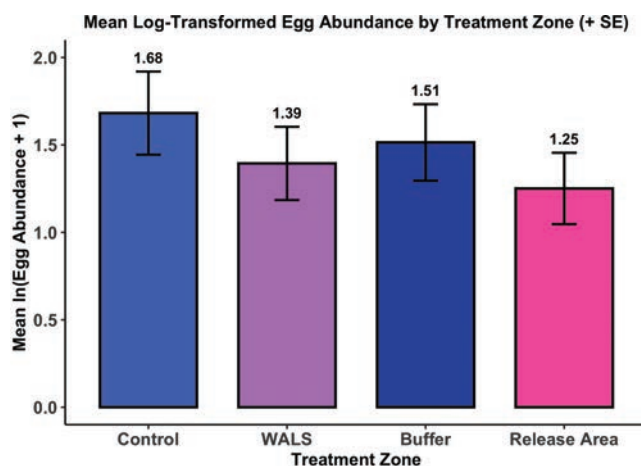


Figure 2.—Mean *Aedes aegypti* egg abundance per treatment zone across the study period. Weekly counts were summed across five ovi-traps within each zone and bars represent raw (untransformed) mean egg counts of these weekly totals. Error bars represent the standard error.

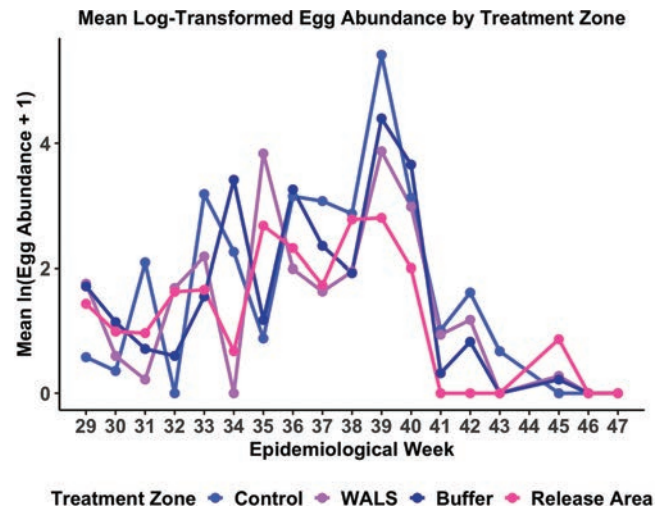


Figure 3.—Weekly *Aedes aegypti* egg abundance by treatment zone across epidemiological weeks 29–47. Mean (untransformed) egg counts represent the sum of eggs collected across five ovi-traps per zone during each sampling week. No trapping was conducted during epi-week 44.

aegypti or females inseminated by released males (Figure 4). Additionally, weekly comparisons showed higher hatch rates during peak mosquito season weeks (epi-weeks 34–35; 37, 39–42) relative to early season baselines (epi-weeks 29–33) (Figure 4).

Discussion

Overall, the results indicated that egg abundance was primarily driven by seasonal variation, whereas hatch rate

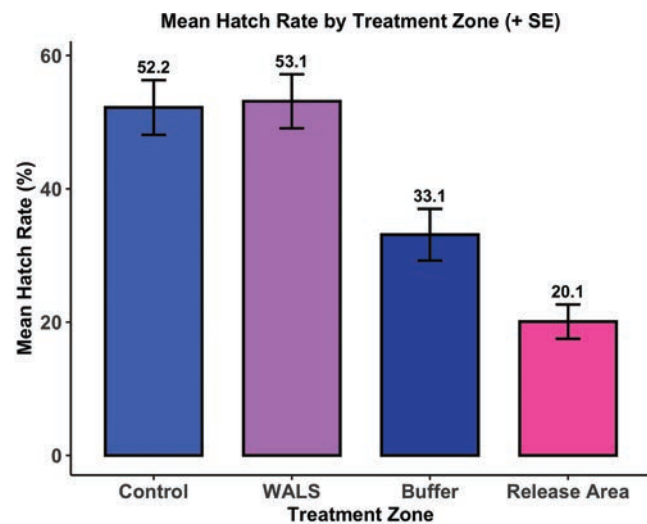


Figure 4.—Weekly *Aedes aegypti* hatch rate (%) by treatment zone across epidemiological weeks 29–45. Hatch rate was calculated as the percentage of successfully emerged individuals relative to viable eggs (total eggs collected minus inviable eggs) across five ovi-traps per zone during each sampling week. No trapping was conducted during epi-week 44 and none of the eggs hatched during epi-weeks 46–47.

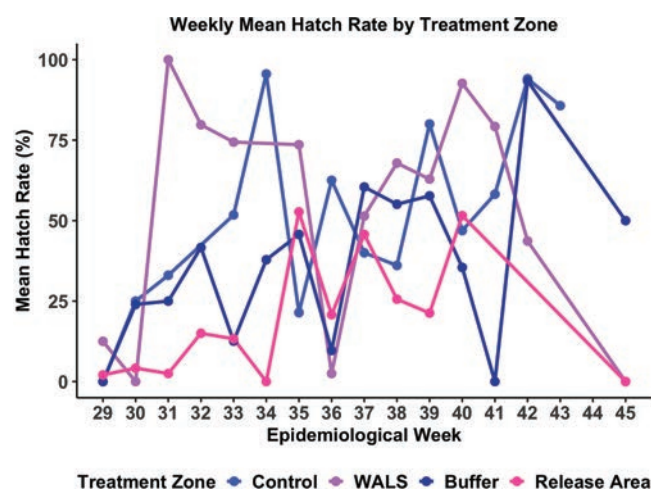


Figure 5.—Mean *Aedes aegypti* hatch rate by treatment zone across the study period. Weekly hatch rates were calculated at the treatment zone level as the percentage of eggs hatched from the total eggs minus inviable eggs collected across five ovitraps per zone and averaged over time. Error bars represent the standard error.

was influenced by both seasonality and treatment zone. Despite the observed reductions in hatch rate, these effects did not translate to proportional declines in egg abundance, likely due to the overriding influence of seasonal adult population dynamics and potential time lags between reduced hatch and subsequent impacts on adult population size. Additionally, IIT primarily affects egg viability rather than oviposition behavior, which may further explain the lack of a direct relationship between hatch rate and egg abundance. Log-transformed egg abundance analyses showed that epi-week was the dominant predictor of oviposition activity, consistent with previous studies demonstrating that environmental factors such as temperature, humidity, and photoperiod regulate *Ae. aegypti* reproductive dynamics, particularly in temperate regions (Becker 2008, Brady et al. 2014). The peak in egg abundance observed around epi-week 39 corresponds with late summer and early fall conditions that favor increased adult survivorship and oviposition activity (Mordecai et al 2019, Sehi et al. 2025).

Although treatment zone was not a significant predictor of egg abundance, consistently lower egg counts were observed in the Release Area relative to the Control. This pattern indicated that combined WALS and IIT applications may have contributed to modest reductions in adult populations; however, these effects were likely masked by seasonal drivers and spatial heterogeneity typical of urban *Ae. aegypti* populations (Harris et al. 2021, Murray et al. 2021). Because WALS targets the immature stages, it does not directly impact adult abundance, which may explain the synchronized oviposition patterns observed across treatment zones (Williams et al 2022). Additionally, the presence of abundant cryptic larval habitats in urban environments may further limit the overall effectiveness of wide area larvicide applications (Barrera 2016).

In contrast, hatch rate differed significantly across treatment zones, highlighting its utility as a sensitive indicator of IIT performance. Reduced hatch rates in both the Release and Buffer zones relative to Control and WALS zones indicated successful mating between wild females and *Wolbachia*-infected males. These findings were consistent with previous studies showing that IIT can reduce egg viability without immediately affecting oviposition output, particularly during early stages of implementation (O'Connor et al. 2012; Turley et al. 2013; Mains et al. 2016; Crawford et al. 2020; Lau et al. 2021). The absence of a significant zone-by-epi-week interaction indicated that temporal trends in hatch rate were similar across zones, whereas overall hatch levels differed by treatment. This pattern indicated sustained reproductive suppression in the Release and Buffer zones throughout the study period.

Additional operational and environmental factors likely contributed to variability in both egg abundance and hatch rate. Trap disturbances from construction, landscaping, or resident activity may have introduced variability in egg counts, a common limitation in residential surveillance (Ritchie et al. 2003). In addition, ovi-trap placement in front yards, combined with skip oviposition behavior and competition from alternative container habitats, may have reduced trap attractiveness (Oliva et al. 2013). Environmental conditions, including rainfall later in the study period, may have further diluted treatment effects by increasing the availability of oviposition habitats. For emergence assays, factors such as egg desiccation, handling, and larval density may have influenced outcomes; however, these effects were consistent across all treatment zones. As a result, the observed reductions in hatch rate in the Release and Buffer zones were most likely attributable to IIT-driven reproductive interference (Hoffmann et al. 2011).

Conclusion

Our study evaluated integrated control strategies for *Ae. aegypti* in the residential neighborhood of South Natomas. Results indicated that egg abundance was largely affected by seasonal trends whereas hatch rate was more responsive to combined IIT and WALS interventions. These findings indicated that integrating WALS-based larval control measures with *Wolbachia*-based male releases may provide complimentary population suppression benefits, with WALS reducing larval development and subsequent adult emergence, and IIT directly reducing egg hatch through cytoplasmic incompatibility. However, egg abundance patterns likely also reflect underlying seasonal population dynamics, with generational increases through late summer followed by declines under less favorable environmental conditions.

As *Ae. aegypti* continues to expand in Sacramento and Yolo counties, ongoing evaluation of integrated approaches will be imperative for improving operational effectiveness and success. Future work should focus on evaluating long-term population suppression, conducting similar studies in

other heavily infested residential communities, and recording environmental variables such as temperature, wind, and humidity, to further examine the effect of control interventions and seasonality's impact on oviposition activity. Overall, these results support the use of combined control strategies to enhance sustainable management of *Ae. aegypti* in urban settings.

Acknowledgements

We want to acknowledge and thank the SYMVCD Urban Control Operations Crew, who assisted with securing the 60 surveillance sites for the study: D. Zepf, C. Reyes, A. Barrera, and A. Misirli. We would also like to extend our sincerest thanks to the residents of South Natomas for their participation in this study.

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William E. Walton Poster Competition

The William E. Walton Poster Competition was initiated in 2023 to recognize exceptional poster presentations in the field of mosquito and vector control research and operations. The annual competition honors the career of the late Dr. William (Bill) E. Walton, Professor of Entomology at the University of California, Riverside, who was passionate about and committed his career to the study of entomology and vector ecology. Each year the number of participants and diversity of topics in the competition has increased and serves as a showcase for innovative research and applied science in vector control and public health.

Posters have long served as an essential platform for presenting early-stage research, introducing methodological innovations, and fostering collaborative dialogue. Previous submissions reflect the remarkable diversity, creativity, and scientific rigor that define our field. Covering a wide range of topics, including operational evaluations of surveillance and control strategies, public outreach initiatives, and research on vectors and vector-borne diseases, these posters underscore the vital role of research and technology in advancing integrated vector management.

First place winners for each year

Year	Presenting Author	Poster title
2026	Christopher Vuong	Reducing Plague Risks in California Recreation Areas: Testing A Modified Bait Tube Design for Improved Flea Control on Sylvatic Rodents
2025	Alexandra Echenberg	Evaluating drone applications of larvicide to rural sites in the Coachella Valley
2024	Brittany Deegan	The impact of aromatic plants on BG Sentinel II trap efficacy
2023	Sumiko De La Vega	Evaluation of a cordless heat gun for ovicidal treatment of <i>Aedes aegypti</i> eggs

Reducing plague risks in California Recreation Areas: Testing a modified bait tube design for improved flea control on sylvatic rodents

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Abstract

Yersinia pestis, the pathogen responsible for plague, is transmitted from rodent hosts to humans primarily through the bites of infected fleas. Two of the primary methods to control rodent fleas include pesticide applications to rodent burrows and insecticide-treated bait tubes, both used to reduce plague transmission and human risk in public use areas such as campgrounds. In some scenarios, the standard, larger-diameter bait tubes do not reduce fleas on smaller rodents such as chipmunks potentially due to the guarding of tubes by larger and more aggressive ground squirrels, namely California ground squirrels (*Otospermophilus beecheyi*). We examined whether modified bait tubes with a smaller diameter and larger bait holding capacity would be more effective at reducing flea prevalence and abundance on smaller rodents by limiting use by larger ground squirrels. The bait tube evaluations took place at public campgrounds in the Lake Tahoe area with the cooperation of the U.S. Forest Service and California State Parks. A before-after, control-impact (BACI) study design was used. For each of 3 replicates, the standard (4-inch diameter) and modified (3-inch diameter) bait tubes were placed in separate 7x7 grids (30-meter spacing). Bait tubes were installed at each treatment site for two weeks and rebaited every four days. Rodent traps were set in a 6x6 grid (20-meter spacing) centered within each treatment site before and 2 weeks after bait tube placement to evaluate flea control. Trap grids were also set at nearby control sites (no insecticide treatments). Sites with modified bait tubes saw a greater decrease (mean percent reduction = 93.7%) in chipmunk flea abundance after two weeks than sites with the standard tubes (mean percent reduction = 52.7%). Sites with modified bait tubes also had a greater reduction in chipmunk flea prevalence, with a reduction of 76.9% compared to sites with the standard tubes which had a mean reduction of 24.4%. Rodent species and site-level flea abundance and prevalence also varied. This study shows the potential for the modified bait tube design to improve chipmunk flea control for plague risk reduction.

Reducing Plague Risk in California Recreation Areas: Testing A Modified Bait Tube Design for Improved Flea Control on Sylvatic Rodents

Christopher T. Vuong¹, Gregory M. Hacker¹, Stefan Sielsch², Mark G. Novak¹

¹ CDPH VBDS, ² El Dorado County Vector Control



Introduction

Yersinia pestis, the etiological agent of plague, is typically transmitted among rodents by fleas. Many recreational areas in California can present increased transmission risk for humans. Carpeted bait tubes treated with insecticides are one method used to control rodent fleas and mitigate plague risk in recreation areas (Bronson and Smith, 2002). Prior experience has shown that the standard bait tubes struggle to control fleas on smaller rodents like chipmunks (*Neotamias* spp.), likely due to dominance by larger California ground squirrels (*Otospermophilus beecheyi*). In this study, we assessed whether a modified bait tube with a smaller diameter was more effective at controlling fleas on chipmunks by excluding larger ground squirrels.

Hypotheses:

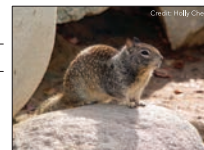
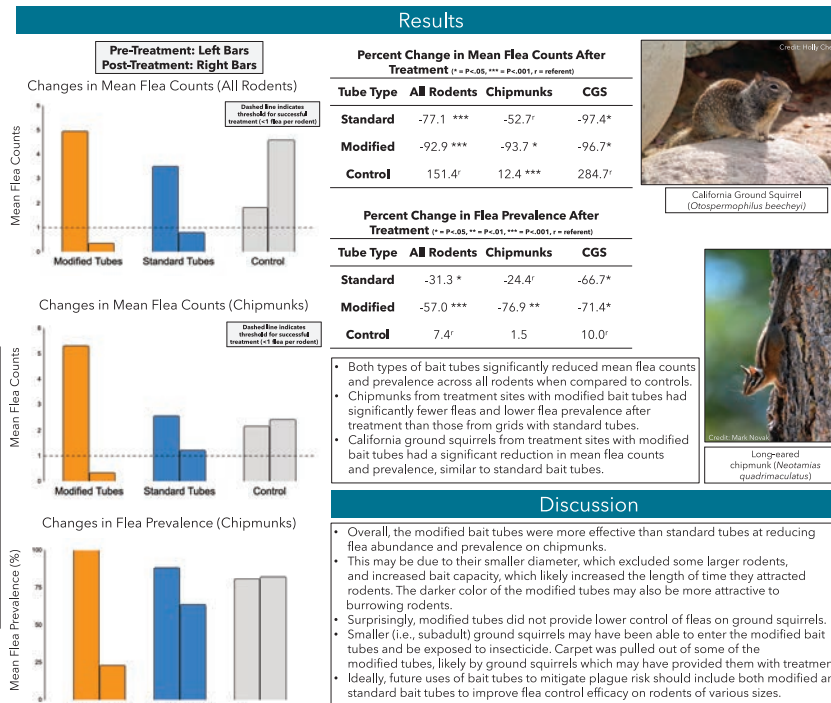
1. The modified bait tubes will be associated with a greater reduction in flea counts and prevalence among chipmunks, compared to standard bait tubes.
2. The modified bait tubes will be associated with a lower reduction in flea counts and prevalence among California ground squirrels (CGS), compared to standard bait tubes.

Methods

- Standard bait tubes (Figure 1) were 4-inch diameter white PVC pipe, while modified bait tubes were made of 3-inch black ABS pipe (Figure 2). The modified tubes had a storage area on top of the tube that increased bait-holding capacity. Detex Blox (Bell Laboratories) were used to bait both tube types and both tube types were lined with carpet sleeves pretreated with 0.06% deltamethrin (Suspend SC, Bayer).
- The flea control efficacy of modified and standard bait tubes were compared against a control (area with no bait tubes). Bait tubes were placed by type (i.e. modified or standard) in separate 3.3-hectare sites, in 7 x 7 grids with 30m spacing (Figure 3). This was repeated at three recreational sites within the Lake Tahoe Basin.
- Rodent trapping was conducted at each treatment and control site one day before bait tubes were placed and after two weeks. Traps were placed in a 6 x 6 grid with 20m spacing (1 hectare). Captured rodents were identified to species and brushed to determine the number of fleas present for each individual.
- Generalized linear mixed models (negative binomial) were used to compare mean flea counts and logistic regression was used to compare flea prevalence (percentage of rodents with at least one flea).



Figure 3: The three study sites within the Lake Tahoe Basin.



California Ground Squirrel (*Otospermophilus beecheyi*)



Long-eared chipmunk (*Neotamias quadrimaculatus*)

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Acknowledgments

This study was made possible with help from staff from CDPH VBDS (Elk Grove and Richmond) and the El Dorado County Vector Control. Our thanks to the California State Parks and United States Forest Service for cooperation and use of their lands.

Testing lithium-ion power banks in modified Reiter-Cummings gravid traps in a desert environment: a comparison of battery types

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Introduction

Monitoring adult mosquito abundance is a critical responsibility of mosquito control districts, and doing so in a standardized manner is essential for comparing mosquito activity and virus transmission across agencies and over time. Within its urban and suburban areas, the Coachella Valley Mosquito and Vector Control District (District) has used Reiter-Cummings gravid traps (Cummings 1992) for more than 25 years. Powered by 6V batteries, these traps are very portable and have been effective at collecting *Culex quinquefasciatus* females, which are then tested for West Nile, St. Louis encephalitis, and western equine encephalomyelitis viruses.

Although 6V batteries are portable, ubiquitous lithium-ion batteries present an opportunity for improved trap design. Dewsnup et al. (2023) published their modifications to the Reiter-Cummings gravid trap to make use of lithium-ion batteries. The District's location has very high day-time temperatures, and such batteries are known to overheat when charging phones. In the current study, we examined whether traps powered by lithium-ion batteries would have a lower collection size during the hottest months due to poor battery performance.

Methods

For 5 weeks (Jul-Aug 2025), 2 gravid traps were set at each of 4 of the District's routine trap locations. Traps were set at least 6.1 m (20 ft.) apart. One trap per pair had a 6V battery (PowerSonic PS-6100) and an AC/DC motor fan, whereas the second trap used a lithium-ion battery (Anker A1263, now discontinued) housed in a modified compartment and a 4" x 4" USB-A connected computer fan (Fig. 1, 2). Each week, the gravid traps and batteries were rotated at their location. Collections were identified to species, sex, and enumerated; comparisons for significant differences were made using ANOVA. The female mosquitoes were pooled for arbovirus testing.

Results

The average number of females collected at each trap location for each battery type is displayed in Figure 3. Most mosquitoes collected were *Cx. quinquefasciatus*, followed by *Ae. aegypti* and *Cx. tarsalis*. The biggest difference in collections occurred at the Cook and Vintage sites where the lithium-ion battery outperformed the 6V battery. However, no significant statistical difference was found in mosquito collections based on battery type (p-value = 0.4). No virus was detected in the mosquito samples.

Conclusions

The 6V batteries and lithium-ion batteries performed similarly, indicating that a change in battery type should have minimal impact on mosquito collections. The District purchased computer fans with a high blade rotation to keep mosquitoes within the collection chamber. The implementation of lithium-ion battery packs means that the use of computer fans results in less electrical work.

Examining the trap collections, the four locations were chosen as sites that have consistent collections during the summer months. One site, Mesquite, had low collections during the current study because wide area larvicide applications were made near this location. The gravid traps can be made in-house. We found the lithium-ion batteries to be lightweight. Discussions with Salt Lake



Figure 1.—Comparison of the 6V battery on the left (PowerSonic PS-6100) and the lithium-ion battery on the right (Anker A1263).

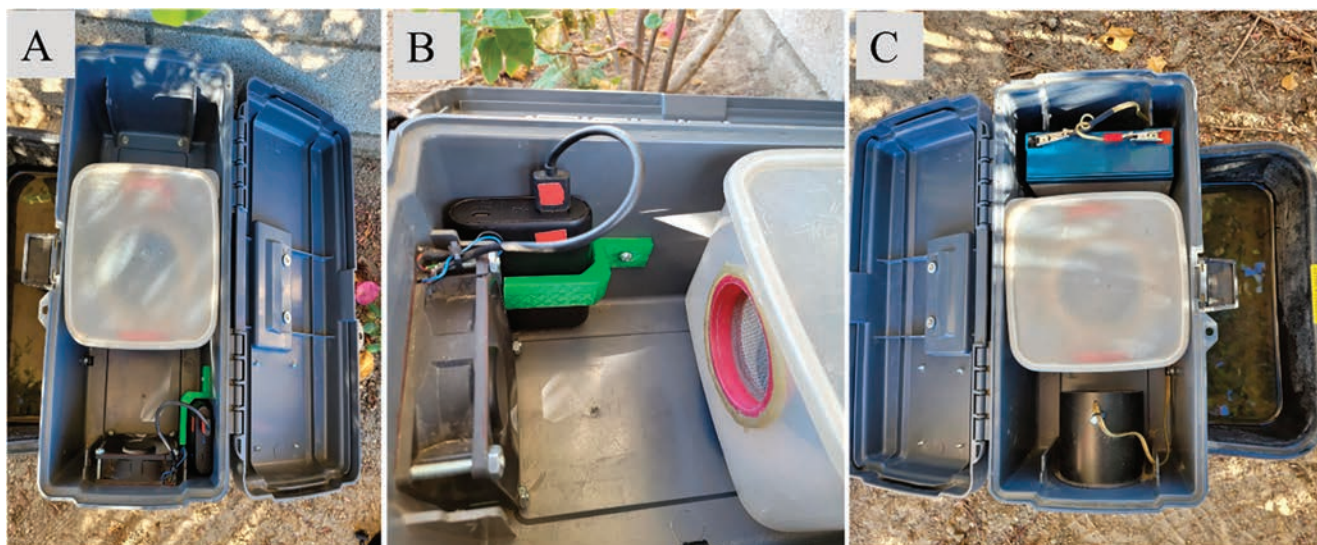


Figure 2.—A. A Reiter-Cummings gravid trap with the lithium-ion battery pack. B. A close up of the lithium-ion battery pack with a 3D printed holder. C. A Reiter-Cummings gravid trap with the 6V battery.

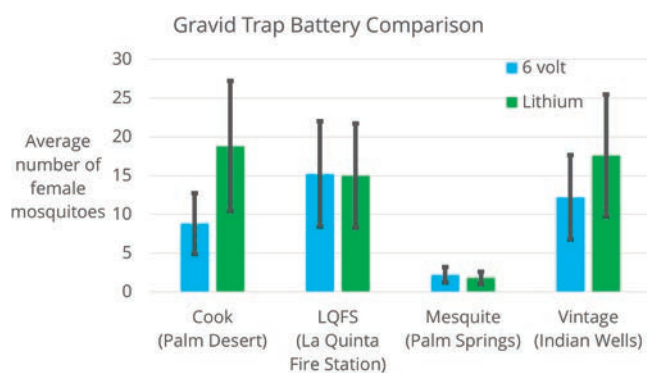


Figure 3.—Comparison of the mean number of female mosquitoes collected per trap night during the five week study period. No significant difference was found between the battery types (ANOVA, $p = 0.4$).

City Mosquito Abatement District indicated a long lifespan may be expected for these batteries, which should provide additional cost savings. We plan to explore the use of this battery type with additional trap types, such as the CO₂ trap and the BG-Sentinel Pro II trap.

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Testing lithium-ion power banks in modified Reiter-Cummings gravid traps in a desert environment: a comparison of battery types

Diana Esparza-Ramirez, BCE, Arturo Gutierrez, Víctor Hernandez, Gabriela Perezchica-Harvey, M.S., and Jennifer A. Henke, M.S., BCE

ABSTRACT: The Coachella Valley Mosquito and Vector Control District (the District) has used Reiter-Cummings gravid traps for more than 25 years to capture female mosquitoes for arbovirus testing. This study compared the performance of 6V batteries and lithium-ion batteries in gravid traps. Overall, no significant difference was found in mosquito collections when using either battery. The District is converting to lithium-ion batteries for their gravid traps in 2026 as they showed comparable effectiveness to 6V batteries.

INTRODUCTION: The District uses Reiter-Cummings gravid traps (Cummings 1992) to collect female *Culex* and *Aedes* mosquitoes for virus testing and surveillance. These traps are powered by 6V batteries. Recently, Salt Lake City Mosquito Abatement District published their work with lithium-ion batteries powering gravid traps (Dewsnup 2023). The District's study explores the performance of lithium-ion batteries and 6V batteries in gravid traps in the Coachella Valley. The findings could show a more effective power source for gravid traps to have consistent mosquito collections.

OBJECTIVE: To use effective, efficient traps with consistent collections of mosquitoes.

METHODS: For 5 weeks (Jul-Aug 2025), 2 gravid traps were set at 4 of the District's routine trap locations. Traps were set 20 ft. apart with one trap using a 6V battery (Figure 1A) and an AC/DC motor fan (Figure 2A). The other trap used a lithium-ion battery (Figure 1B) housed in a modified compartment and a 4" x 4" computer fan (Figure 3A, 3B). Each week, the gravid traps were rotated at their location. Collections were identified to species, sex, and enumerated. The female mosquitoes were pooled for arbovirus testing.



Figure 1. — Battery Types.
A) 6V battery.
B) Lithium-ion power bank with USB-A connection.

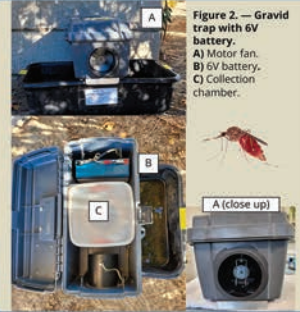


Figure 2. — Gravid trap with 6V battery.
A) Motor fan.
B) 6V battery.
C) Collection chamber.
A (close up).

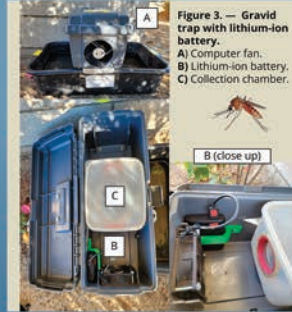
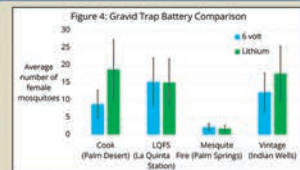


Figure 3. — Gravid trap with lithium-ion battery.
A) Computer fan.
B) Lithium-ion battery.
C) Collection chamber.
B (close up).

RESULTS: The total mosquito collections were counted for each battery type (Table 1). Most mosquitoes collected were *Cx. quinquefasciatus* followed by *Ae. aegypti* and *Cx. tarsalis*. The average number of females collected at each trap location for each battery type is displayed on Figure 4. The biggest difference in collections occurred at the Cook and Vintage sites where the lithium-ion battery outperformed the 6V battery. However, no significant statistical difference was found in mosquito collections based on battery type (p-value = 0.4).

CONCLUSIONS:

- The 6V batteries and lithium-ion batteries performed similarly.
- The District conducted larvicide missions in Palm Springs during the study, which may explain the low collections at Mesquite.
- Gravid traps can be made in-house. Wiring the computer fan takes some additional minor work.
- The District will implement the use of lithium-ion batteries in their traps due to their lightweight flexibility, cost effectiveness, and longer battery life.
- Further studies could explore the effectiveness of lithium-ion batteries on BG-Sentinel traps.



	Lithium Battery	Gravid Females	Gorged Females	Empty Females	Males
<i>Aedes aegypti</i>	3	0	5	2	
<i>Culex quinquefasciatus</i>	158	7	93	194	
<i>Culex tarsalis</i>	0	0	0	1	
Total	161	7	98	197	
	6 Volt Battery	Gravid Females	Gorged Females	Empty Females	Males
<i>Aedes aegypti</i>	6	0	5	2	
<i>Culex quinquefasciatus</i>	132	3	45	139	
<i>Culex tarsalis</i>	0	0	1	0	
Total	138	3	51	141	

Table 1. — Gravid trap total mosquito collections by battery type, July-August 2025, Coachella Valley, CA.

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Mosquito photos from Pngtree, Azelis, Bradley Burbalough, Vinisouza128, Salvador Vitanza, and Arinspiredencil.

For further information, contact Diana Ramirez at the Coachella Valley Mosquito and Vector Control District at dramirez@cvmosquito.org

Determining the prevalence of dog heartworm (*Dirofilaria immitis*) and potential vectors from dog parks in Sacramento and Yolo Counties, CA

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Abstract

Dog heartworm (*Dirofilaria immitis*) is a mosquito-borne filarial nematode that primarily affects domestic dogs, causing potentially fatal cardiopulmonary disease. Transmission occurs during mosquito blood feeding, but which mosquitoes serve as the primary vectors in the Sacramento-Yolo region of California remains unknown. This study aimed to assess the prevalence of *D. immitis* in mosquitoes collected from dog parks and to identify the species most associated with infection. Mosquitoes were collected by Sacramento-Yolo Vector Control District, pooled and screened for *D. immitis* DNA using a polymerase chain reaction (PCR). Preliminary analysis showed that of the 129 mosquito pools tested, 6 were confirmed positive for *D. immitis* DNA. PCR products were visualized by gel electrophoresis, with potential positives indicated by the presence of a ~193-base pair (bp) band, the expected product size for *D. immitis*. These amplicons were purified and submitted for Sanger sequencing for confirmation. Results will be shared with the Sacramento-Yolo Vector Control District. These findings will help identify key vector species contributing to regional transmission of this parasite.

Larval abundance decreases as cemeteries increase adherence to district recommendations

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Abstract

In 2024, the Contra Costa Mosquito and Vector Control District made recommendations to reduce mosquito production at 11 cemeteries, including drilling holes in vases or filling them with dirt or sand, using artificial flowers, and maintaining proper drainage after irrigation. In July 2025, the 11 cemeteries were assigned a compliance score of 0 to 3 based on their adherence to the recommendations, with 0 being incomplete adherence and 3 being complete and total adherence. Over a five week period in July and August 2025, larvae and adults from each cemetery were collected and identified to species. Adults were collected weekly at each cemetery using BG Sentinel traps with dry ice and BG lures. Larvae were collected twice at three sites per cemetery over the same five week period. Across the sites, larval abundance decreased with increased compliance to district recommendations, such that there were fewer mosquito larvae at sites with better compliance. Our poster reviewed the correlation of adherence score to larval and adult abundance.

Body size variation affects permethrin susceptibility in *Aedes aegypti*

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Abstract

Mosquitoes, especially *Aedes aegypti*, are highly invasive in the United States and serve as primary vectors of arboviruses causing diseases such as dengue, Zika, and chikungunya. Current control methods rely heavily on pyrethroid insecticides such as permethrin, cypermethrin, etc.

However, their effectiveness is declining due to insecticide resistance and concerns over environmental impacts from increased usage. Most resistance studies treat mosquito populations as homogeneous, overlooking individual variation in traits such as body mass that may influence susceptibility. Our research addresses this gap by testing how body mass affects permethrin resistance in *Ae. aegypti*. Using both laboratory (Rockefeller strain) and field-derived (susceptible, resistant, Kaohsiung) strains, adult body mass was manipulated by altering larval rearing conditions. Lower densities yielded heavier adults, higher densities produced lighter ones. These mass-variant adults were exposed to permethrin via CDC bottle bioassay. Heavier mosquitoes were less susceptible than lighter individuals, indicating body size played a role in resistance.

Currently, recovery assays simulating sublethal exposure are being done, with mosquitoes exposed for short intervals (1 and 2.5 minutes), which are then monitored for daily mortality. This approach reflects real-world conditions and will shed light on post-exposure recovery dynamics. Overall, this research highlights how individual-level traits like body mass influence insecticide resistance, with implications for designing more targeted and sustainable mosquito control strategies.



BODY SIZE VARIATION AFFECTS PERMETHRIN SUSCEPTIBILITY IN *AEDES AEGYPTI*

Jordan Reyes and Karthikeyan Chandrasegaran

Department of Entomology, College of Natural and Agricultural Sciences, University of California, Riverside



Introduction

Pyrethroid resistance in *Aedes aegypti* is increasingly limiting the operational performance of adulticide applications, including products formulated with permethrin, which can lead to reduced knockdown and spray failures, allowing more mosquitoes to survive control efforts and sustain transmission risk [1]. While resistance is often characterized at the population level, field control outcomes are also shaped by individual heterogeneity [2]. One under examined source of heterogeneity is adult body size, which can alter dose per unit mass and may covary with resistance-linked traits, such as cuticular penetration and detoxification capacity, making these hypothesized mechanisms for size-dependent permethrin outcomes [2]. As a result, mosquitoes are not exposed to insecticides as a uniform group, and body-size assumptions may obscure meaningful variation in susceptibility and downstream life-history consequences [3]. Standard bioassays typically expose cohorts to a fixed dose/time and interpret a single knockdown/mortality endpoint as a representative of the population; my study challenges that assumption by testing whether body size shifts sublethal permethrin effects on survival and fecundity.

In operational settings, sublethal exposure can arise from environmental heterogeneity (uneven spraying, aging residues, shorter/inconsistent contact with treated surfaces) and behavioral heterogeneity (avoidance or reduced resting/contact) [2]. This contrasts with standardized laboratory diagnostic-dose/time assays, which assume uniform exposure to a fixed concentration and duration [2]. Sublethal exposure is significant because it can both alter immediate outcomes, such as recovery and survival, and impose delayed or carryover effects on fitness, including reproduction [4]. However, most sublethal pyrethroid studies do not explicitly stratify or model outcomes by adult body size, leaving it unclear whether size systematically modifies survival and reproductive carryover effects.

This study tests the hypothesis that body size modifies sublethal permethrin outcomes in *Aedes aegypti*, and that the magnitude and direction of size dependence differ by resistance status, reflecting differences in underlying resistance physiology. We manipulate adult body mass by rearing larvae at low density (larger adults) versus high density (smaller adults) and quantify post-exposure survival in both sexes and reproductive output in females. By explicitly incorporating body size into sublethal permethrin response, this research aims to clarify how size-dependent variation shapes phenotypic survival and fecundity, improving the interpretation of resistance bioassays and helping predict when operational applications may underperform under heterogeneous exposure.

Objectives:

- Quantify the relationship between adult body mass and permethrin susceptibility across diagnostic and sublethal exposure levels.
- Evaluate whether size dependent susceptibility patterns differ between field derived susceptible and resistant genetic backgrounds.

Experimental Approach

Aedes Aegypti Strains:
Lab susceptible: Rockefeller (ROCK)
Field susceptible: Kaohsiung (KS)
Field-derived: Susceptible (SUS) Resistant (RES)

Figure 1: Low Density (LD) vs. High Density (HD)

Figure 2: CDC Bottle Bioassay

Figure 3: Recovery Assay

Results

Figure 4: Bottle Bioassay Mosquito Statuses Over Time Across Dosages

Figure 5: Recovery Assay Mosquito Knockdown After 1 Hour (dd)

Results

Figure 6: Recovery Assay Mosquito Survival Post-Exposure (dd)

Future Directions

- Thermal tolerance assays:** Expose sublethal permethrin treated mosquitoes to controlled heat and cold ramping assays to quantify size and resistance dependent shifts in critical thermal limits, knockdown thresholds, and survival trajectories. This will allow estimation of CTmax, CTmin, and lethal time metrics to test whether insecticide exposure alters thermal resilience in a size dependent and genotype dependent manner.
- Longitudinal activity monitoring:** Monitor sublethal permethrin treated mosquitoes using automated actometers to quantify circadian and diel activity patterns, locomotor performance, and recovery dynamics across size classes and resistance backgrounds. Behavioral metrics will include total activity, temporal activity structure, and post exposure impairment or recovery trajectories.
- Blood feeding and reproduction assays:** Subject sublethal permethrin treated females to standardized blood feeding and oviposition assays to quantify effects on feeding propensity, blood meal size, egg production, and oviposition behavior. These assays will test whether body size and resistance status modulate sublethal carryover effects on reproductive output and behavioral decision making.

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UC RIVERSIDE Department of Entomology

Standardizing SmartWater[®] as a marking method for *Aedes aegypti*

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Abstract

Mosquitoes are important vectors of arboviruses, and control strategies such as the Sterile Insect Technique(SIT) rely on reliable marking methods to assess the survivorship and dispersal of irradiated males released in the field. Existing marking methods are often only partly effective or incur recurring operational costs, highlighting the need for an efficient, cost-effective alternative. To address this gap, we repurposed and standardized SmartWater[®], a polymer-based dye that fluoresces under specific UV wavelengths. Although prior research has explored SmartWater[®] for *Anopheles* mosquitoes, little is known about its performance in other species or its persistence under environmental stressors, such as sunlight exposure. In this study, we used three detection methods, namely, visual classification, GelDoc imaging, and plate reader-based quantification, to evaluate dye persistence and durability under sunlight exposure. The dye itself did not significantly affect mosquito survivorship. However, the choice of dye color(fluorophore) influenced detection performance, indicating that dye color plays an important role in the reliability of mark identification. Because marking approaches used in SIT programs must not interfere with mosquito fitness or mating success, we also evaluated whether SmartWater[®] affects male reproductive performance. We compared unmarked and marked male mosquitoes by quantifying the number of eggs and viable offspring produced by cohabiting unmarked female mosquitoes. Male mosquitoes treated with SmartWater[®] showed no detectable reduction in reproductive success compared with unmarked male mosquitoes. Overall, these results demonstrate that SmartWater[®] represents an efficient, durable, and cost-effective alternative to existing marking strategies for SIT and related mark-release-recapture applications.



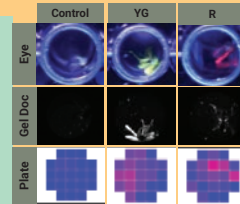
Assessing SmartTrace® for Mark–Release–Recapture in Sterile Insect Technique Programs

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Department of Entomology, University of California Riverside, Riverside CA



Introduction:

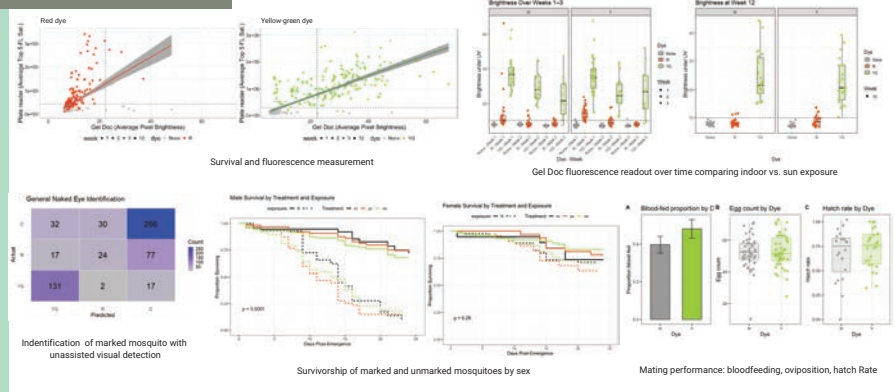
Mosquitoes are major disease vectors worldwide. *Aedes aegypti*, an invasive mosquito species established in many regions is the primary vector of dengue, chikungunya, and Zika viruses. There are no widely available vaccines for chikungunya, and while vaccines exist for dengue and Zika, they are either not widely accessible or not the primary approach. As a result, mosquito population control remains the main strategy for preventing disease transmission. A common approach to vector control is the Sterile Insect Technique (SIT), which has minimal off-target effects. SIT involves releasing sterilized male mosquitoes (via X-ray exposure) that compete with wild males for mating, resulting in sterile eggs. The success of SIT depends on the ability to track mosquito survival, dispersal, and mating success using mark-release-recapture (MRR) methods. Current marking tools include fluorescent powders, which are easy to detect but lack long-term persistence, and stable isotopes or egg albumin, which have high recurring detection costs. To address these limitations, we evaluated SmartTrace®, a polymer-based fluorescent dye that comes in six colors and is detectable under specific wavelengths of UV light. Although SmartTrace® has been tested in *Anopheles*, it has not been evaluated in *Ae. aegypti*, nor has its fluorescence or persistence been quantified. **In this study, we compared three detection methods (Gel Doc, plate reader, and visual detection) and tested dye persistence under laboratory and field conditions. We also assessed whether marking affected mosquito survival and male mating performance, measured through female blood feeding, egg laying, and egg hatch.**



Method:



Result:



Work Cited:

Falman R, et al. A novel fluorescence and DNA combination for versatile, long-term marking of mosquitoes. *Methods Ecol. Evol.* 2021 Jun;12(6):1008-1016. doi: 10.1111/2041-210X.13592.
Birhanie SK, et al. Preparation for targeted sterile insect technique to control invasive *Aedes aegypti* (Diptera: Culicidae) in southern California: dose-dependent response, survivorship, and competitiveness. *J Med Entomol.* 2024 Nov 14;61(6):1420-1426. doi: 10.1093/jme/tjae106.

Conclusion:

All three detection methods successfully detected the dye, with yellow-green showing consistently stronger performance. Marking didn't noticeably affect survival in either sex, even under sun exposure, compared to sun-exposed unmarked mosquitoes. Mating with marked individuals did not affect blood feeding, egg laying, or egg hatch rate. Together, these results indicate that SmartTrace® is a strong alternative to existing marking methods providing durable detection without affecting their survival or reproduction, making it suitable for Mark-Release-Recapture in SIT programs.



Video of marked mosquitoes

Acknowledgement:

This research was generated from the Pacific Southwest Center of Excellence in Vector-Borne Diseases. This research was generated from the Pacific Southwest Center of Excellence in Vector-Borne Diseases, which has been funded by the cooperative agreement [U01CK000516 and/or U01CK000649, as applicable] from the Centers for Disease Control and Prevention. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention. We would like to thank the support from Dr. Kerry Mauck, Dr. Daiki Fujinaga, Dr. Naoki Yamanaka, Imad M Bayoun, Maria Mendiola, Joseph Akolgo, Monique Velasco, and Elspeth Cannell.

Small sprays for smaller vectors: Effects of ULV sprays on *Culicoides* populations in Lake County CA

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The *Culicoides* biting midge is a small biting fly, several species of which are implicated in transmitting parasites and viruses of human and animal importance. There are approximately 60 species documented in California, including *C. sonorensis*, which is known to transmit bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV). Despite this, control strategies are understudied. *Culicoides* have been shown to be susceptible to permethrin in the lab, and Ultra Low Volume (ULV) sprays using permethrin as their active ingredient have been shown to be effective in semi-field cage trials. While a common tool for some vector control districts, their efficacy in biting midge control has not been evaluated in field settings. This study evaluated the effects of ULV sprays on

Culicoides populations across Lake County, California. Routine sprays were performed at three sites weekly from July to October 2025, and midges were collected using miniature CDC light traps twice each week (pre-spray and post-spray) and identified to species. Targeted sprays were also performed at three sites at dawn to test the effect of ULV sprays during higher rates of adult midge activity. Just as with the routine sprays, midges were collected twice weekly using miniature CDC light traps and identified to species. Effects of ULV sprays on *Culicoides* abundance at different times compared to control sites are presented, as well as data on seasonal abundance of Lake County's biting midge species. This study provides valuable data and practical considerations for use of ULV sprays as control for *Culicoides* in California.

Evaluating Sumilarv use for Santa Cruz catch basins

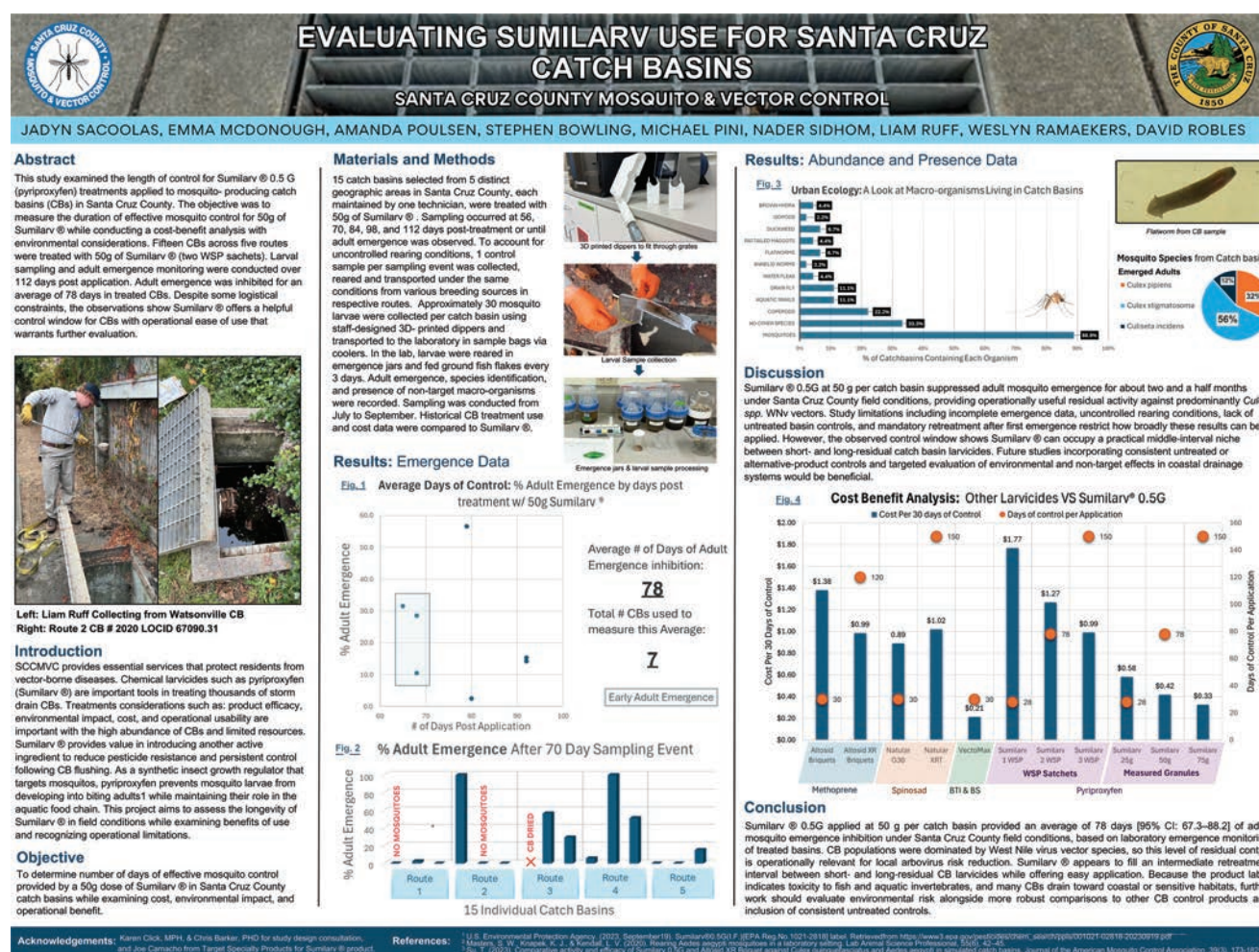
Jadyn Sacoolas, Emma McDonough*, Amanda Poulsen, Stephen Bowling, Michael Pini, Nader Sidhom, Liam Ruff, Weslyn Ramaekers, and David Robles

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Abstract

This study examined the length of control for Sumilarv® 0.5 G (pyriproxyfen) treatments applied to mosquito-producing catch basins (CBs) in Santa Cruz County. The objective was to measure the duration of effective mosquito control for 50g of Sumilarv® while conducting a cost-benefit analysis with environmental considerations. Fifteen CBs across five routes were treated with 50g of Sumilarv® (two WSP sachets). Larval sampling and adult emergence monitoring were conducted over 112 days post application. Adult emergence was inhibited for an average of 78 days in treated CBs. Despite some logistical constraints, the observations show Sumilarv® offers a helpful control window for CBs with operational ease of use that warrants further evaluation.



Transgenerational effects of Roundup® exposure on *Culex quinquefasciatus* and its impact on Bti susceptibility, development, and reproductive Fitness

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Introduction

Culex quinquefasciatus is the primary vector of West Nile virus in southern California. Herbicides such as glyphosate-based formulations (e.g., Roundup®) frequently contaminate residential water systems, agricultural runoff, and urban stormwater structures where immature mosquitoes develop. Although *Bacillus thuringiensis israelensis* (Bti) remains a cornerstone larvicide in mosquito control, the potential long-term ecological interactions between chronic glyphosate exposure and Bti susceptibility remain insufficiently studied. Understanding transgenerational herbicide effects on mosquito biology may therefore be critical for predicting operational impact and ensuring sustained field efficacy of core mosquito control tools.

Materials and Methods

A laboratory colony of *Cx. quinquefasciatus* was exposed to 1 mg/l Roundup® only during larval development across six sequential generations (F1-F6). In the 7th generation (F7), the 4th instar larvae (L4) were subjected to Bti (1 mg/l) with and without Roundup® co-presence to determine whether Roundup® legacy exposure increased susceptibility to biological larviciding. Multiple biological endpoints reflecting vector production and life history fitness were subsequently analyzed including larval survivorship, pupal emergence pattern over time, adult morphometric indices (weight, length, wingspan), and fecundity measured by number of egg rafts produced per generation and the successful rate of egg hatching. Transgenerational population level pupal emergence was further quantified across daily emergence curves in all six generations. Statistical analyses included two-way mixed ANOVA, GEE Poisson modeling, Holm corrected post-hoc contrasts, Hedges g effect sizes, and parametric assumption diagnostics.

Results

Roundup®-derived lineage history significantly increased susceptibility to Bti challenge in the 7th generation, resulting in reduced larval survival relative to controls at the same Bti dose. Across the six transgenerational cycles, Roundup® exposure significantly altered daily pupal emergence dynamics, with GEE modeling indicating multiple days per generation in F1-F4 showing significantly lower emergence relative to controls following Holm correction. Total pupal productivity per pan (measured cumulatively per generation) revealed a Treatment × Generation interaction (two-way mixed ANOVA), with Roundup® lineages producing progressively fewer pupae by F5-F6 compared to controls. Adult morphometrics demonstrated reductions in body weight, but no change was observed in body length and wingspan across later generations in Roundup® exposed lineages, indicating developmental fitness cost accrual over time. Finally, fecundity (egg raft production) significantly declined across successive generations in Roundup® exposed mosquitoes compared to controls.

Conclusion

These findings demonstrated that chronic, transgenerational exposure to Roundup® induces significant cumulative biological costs in *Cx. quinquefasciatus* affecting larval survival under Bti challenge, development, emergence timing, morphological fitness, and reproductive output. This work emphasizes the need for vector control agencies to consider herbicide contamination as an emerging ecological modifier of mosquito population structure and control vulnerability when evaluating long-term Bti field performance and integrated management strategy reliability in urban/agricultural California landscapes.



Transgenerational Effects of Roundup® Exposure on *Culex quinquefasciatus* and Its Impact on Bti Susceptibility, Development and Reproductive Fitness

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Department of Health Science and Human Ecology, California State University, San Bernardino, CA



Introduction

Widespread agricultural use of glyphosate, the active ingredient in Roundup®, results in frequent runoff into stagnant water bodies that serve as mosquito breeding sites. This study investigates whether prolonged glyphosate exposure across multiple generations of *Culex quinquefasciatus* alters larval sensitivity to *Bacillus thuringiensis israelensis* (Bti), a widely used microbial larvicide.

Materials & Methods

Conditions: Temperature, humidity, and photoperiod were maintained at $27 \pm 2^\circ\text{C}$, $75 \pm 5\%$, and 12L:12D respectively
Colony maintenance: Larvae were reared in cups filled with 200 mL distilled water and fed every three days.
 This study investigates whether prolonged glyphosate exposure across multiple generations of *Culex quinquefasciatus* alters larval sensitivity to *Bacillus thuringiensis israelensis* (Bti), a widely used microbial larvicide. Mosquito colonies were maintained for six generations in water containing 1 mg/l Roundup®. In the seventh generation, first- and fourth-instar larvae were exposed to 0.1 mg/l Bti, with or without concurrent glyphosate exposure.



Fig. 1: Larval experiment setup (50 larvae in glyphosate solution vs. water)



Fig. 2: female adult measurements



Fig. 3: oviposition cups (control vs. treatment)

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Results

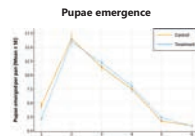


Fig. 4: Daily pupal emergence varied significantly across the 6-day emergence period (Wald $\chi^2(5)=1141.46$, $p < 0.0001$). (Wald $\chi^2(1)=12.86$, $p=0.045$). The Group $\times$ Day interaction approached but did not reach conventional statistical significance (Wald $\chi^2(5)=10.12$, $p=0.072$).



Fig. 5: When total pupal emergence was summed across all six days per pan, no significant difference was detected between Control (mean $\pm$ SD = 42.65 ± 6.44) and Treatment (41.33 ± 7.03) groups (Welch's $t(117)=1.07$, $p=0.287$; 95% CI for difference: -1.12 to 3.76; Cohen's $d=0.20$). A Mann-Whitney U test similarly showed no significant difference between groups ($U=1920.5$, $p=0.527$).

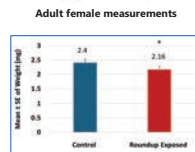


Fig. 6: Mean $\pm$ SE of weight (mg) of emerged adults from transgenerationally exposed larvae of *Culex quinquefasciatus* to Roundup® versus control adults (F1-F6). Shapiro-Wilk test of normality: $W = 0.857$, $p = 0.179$ (no evidence of non-normality). Paired sample t-test, $n = 10$, $df = 9$, $t = 4.021$, $P < 0.05$. Mean values are indicated on each bar; An asterisk on top of a bar indicates a statistically significant difference at 95% interval confidence.

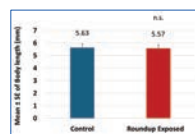


Fig. 7: Mean $\pm$ SE of body length (mm) of emerged adults from transgenerationally exposed larvae of *Culex quinquefasciatus* to Roundup® versus control adults (F1-F6). Shapiro-Wilk test of normality: $W = 0.937$, $p = 0.633$ (no evidence of non-normality); Paired sample t-test, $n = 10$, $df = 9$, $t = 0.829$, $P > 0.05$. Mean values are indicated on each bar; n.s.: Non-significant difference.

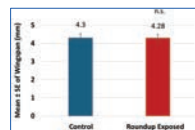


Fig. 8: Mean $\pm$ SE of wingspan (mm) of emerged adults from transgenerationally exposed larvae of *Culex quinquefasciatus* to Roundup® versus control adults (F1-F6). Shapiro-Wilk test of normality: $W = 0.776$, $p = 0.035$ (data do not have a normal distribution); Wilcoxon signed-rank test, $n = 10$, $df = 9$, $W = 7.0$, $P = 0.892$. Mean values are indicated on each bar; n.s.: Non-significant difference.

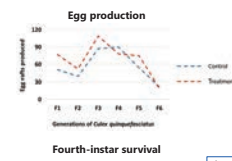


Fig. 9: Egg raft production in *Culex quinquefasciatus* across generations. Paired sample t-test: $t(5)=1.78$, $P=0.134$. Effect size (Cohen's d) = 0.73, 95% CI (-27.25, 4.92).

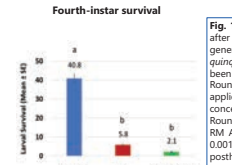


Fig. 10: Mean $\pm$ SE of the larval survival after being treated with Bti on the 7th generation larvae of *Culex quinquefasciatus*, transgenerationally been exposed to the herbicide Roundup® at their immature stages. Bti applied to the 4th instar larvae at 1 mg/l concentration with and without Roundup® presence. RM ANOVA: $n = 10$, $F(2, 18) = 74.22$, $p < 0.001$, partial $\eta^2 = 0.892$. Tukey HSD posthoc test. Mean values are indicated on each bar; Different letters on each bar indicates statistically significant differences. Bti: *Bacillus thuringiensis israelensis*.

Conclusion

Glyphosate-exposed *Culex quinquefasciatus* showed susceptibility to Bti and a correlation with adult female weight. This study did not find a strong link between generational glyphosate exposure and pupae emergence, adult female size, and egg production. Differences in susceptibility between Bti and the Bti-Roundup® solution were not significant.

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Wrangling right-hand drive Jeeps into a better catch basin program

Mark Wieland* and Ryan Clausnitzer

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*Corresponding author: markw@mosquitoes.org

Abstract

We describe our adaptation of a leased 2025 right-hand drive Jeep Wrangler for use in our catch basin program. Our focus was to minimize unnecessary drilling by conforming to existing factory mounts while keeping the functionality simple and clean. By utilizing upgraded available accessory storage compartments, we eliminated the need for awkward and vision obstructing plastic driver-chemical dividers. The four-door configuration and rear-seat organizers provided additional storage with easy access to safety equipment, public outreach materials, and personal or seasonal gear. In addition, modified and custom-built brackets for spray bottles, trigger wands, and dry chemicals contributed to this ergonomic version.

Every catch basin program is different. Understand what works for your District and research after-market accessory retailers first to find adaptable brackets, containment, and storage options before spending unnecessary funds on custom fabricated components. Not everything has to be custom made.



Wrangling Wranglers: Wrangling Right-Hand Drive Jeeps into a Catch Basin Workhorse

Mark Wieland, Ryan Clausnitzer

Alameda County Mosquito Abatement District, 23187 Connecticut Street, Hayward, CA 94545, USA

STORAGE



A custom tailgate mounted shelf secures a 10-gallon spot-sprayer. Weather-proof, sealed storage boxes add to safety, accessibility, and separation from the operator, eliminating the need for costly and vision obstructing dividers.

OPTIONS



HANDS ON



Modified door hinge step brackets connect to custom accessory boxes to hold hand-cans and trigger sprayers. Catch-basins are treated with liquid and/or dry products from the ease of a multi-function supply box. Security, safety, and accessibility are always the goal.

CONTAINMENT



All-weather rear storage creates a containment area adequate for secondary storage containers and a 10-gallon spot-sprayer. Removal of the tailgate panel increases ventilation and allows easy routing for wiring and plumbing. No drilling necessary!

CARGO



The available four-door option provides ample storage and easy access for safety, public outreach, personal and seasonal gear, and other non-hazardous items.

MULTI-PURPOSE



Modified ammo-boxes provide an additional layer of containment. Optional products are stored and available to the operator from the rear containment storage.

Assessing the prevalence of *kdr* mutations linked to pyrethroid resistance in *Culex tarsalis* populations near Sacramento, California

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Abstract

Culex tarsalis is a primary vector for West Nile and St. Louis encephalitis viruses in California, where pyrethroid insecticides remain a primary tool for adult mosquito control. Pyrethrins target voltage-gated sodium channels (VGSCs) in mosquito nerve cells, causing prolonged channel opening and hyperexcitation that leads to paralysis and death. However, point mutations in the VGSC gene can alter the channel's structure, reducing the binding affinity of pyrethrin-based insecticides and resulting in decreased sensitivity. This knockdown resistance (*kdr*) has become an increasing concern for long-term efficacy of mosquito control programs across California. Our study investigated the prevalence of knockdown resistance (*kdr*) mutations in *Cx. tarsalis* populations collected from Conaway and Vic Fazio between July and September 2025. Genomic DNA was extracted using Qiagen DNeasy® Blood & Tissue Kit, and allele-specific quantitative PCR (qPCR) with melt-curve analysis was used to detect substitutions at the *kdr* locus, where leucine (L) is replaced by phenylalanine (F) or serine (S), conferring target-site insensitivity to pyrethroids. Samples with ambiguous melt peaks were identified by Sanger sequencing, with an additional 5% samples sequenced for confirmation. Preliminary results show that most individuals carried the F allele, with a smaller proportion of F/S heterozygotes and no wild-type (L/L) genotypes detected. These findings indicated a near fixation of the F allele in these *Cx. tarsalis* populations and emphasized the need for continued monitoring to support effective vector control operations in Sacramento and Yolo Counties, CA.

Assessing the Prevalence of *kdr* Mutations Linked to Pyrethroid Resistance in *Culex tarsalis* Populations Near Sacramento, California

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ABSTRACT

Culex tarsalis is a primary vector for West Nile virus and St. Louis encephalitis viruses in California, where pyrethroid insecticides remain a primary tool for adult mosquito control. Pyrethrins target voltage-gated sodium channels (VGSCs) in mosquito nerve cells, causing prolonged channel opening and hyperexcitation that lead to paralysis and death. However, point mutations in the VGSC gene can alter the channel's structure, reducing the binding affinity of pyrethrin-based insecticides resulting in decreased sensitivity. This knockdown resistance (*kdr*) is an increasing concern for long-term efficacy of mosquito control programs across California. This study investigates the prevalence of knockdown resistance (*kdr*) mutations in *Cx. tarsalis* populations collected from Conway and Vic Fazio between July and September 2025. Genomic DNA was extracted using Qiagen DNeasy® Blood & Tissue Kit, and allele-specific quantitative PCR (qPCR). Melt-curve analysis was used to detect substitutions at the *kdr* locus, where leucine (L) is replaced by phenylalanine (F) or serine (S), conferring target-site insensitivity to pyrethroids. Samples with ambiguous melt peaks were identified by Sanger sequencing, with an additional 5% samples sequenced for confirmation. Preliminary results show that most individuals carried the F allele, with a smaller proportion of F/S heterozygotes and no wild-type (L/L) genotypes detected. These findings suggest a near fixation of the F allele in these *Cx. tarsalis* populations which emphasize the need for continued monitoring to support effective vector control operations in Sacramento and Yolo Counties, CA.

INTRODUCTION

- *Culex tarsalis* is a primary vector of West Nile virus and St. Louis encephalitis virus in California¹.
- Although typically associated with rural and agricultural habitats, *Cx. tarsalis* is a strong flyer and disperser, and is frequently encountered in urban environments².
- Mosquito and vector control programs rely heavily on pyrethroid insecticides for rapid knockdown of adult mosquitoes to break the transmission cycle^{3,4}.
- Pyrethroids target voltage-gated sodium channels (VGSCs) in the mosquito nervous system. Repeated or sustained use of these insecticides can exert selection pressure which favors individuals carrying mutations associated with reduced sensitivity⁵.
- One well-characterized resistance mechanism is knockdown resistance (*kdr*), which is caused by point mutations in the VGSC Gene that reduce pyrethroid binding affinity⁶.

OBJECTIVES

- Assess the prevalence of *kdr* mutations associated with pyrethroid resistance in *Culex tarsalis* populations collected near Sacramento, California.
- Identify the distribution of resistance-associated alleles phenylalanine (F), serine (S), and wild-type leucine (L) across sample sites and collection months.

METHODOLOGY

Mosquito Collection:

- Adult *Culex tarsalis* mosquitoes were collected by the Sacramento-Yolo Mosquito & Vector Control District (Sac-Yolo MVCD) using CO₂ traps placed throughout designated rice field locations in the Yolo Bypass area near Sacramento, California.
- CO₂-baited traps were placed at two sites: Conway (control site) and Vic Fazio Wildlife Area (pyrethroid-treated site).
- Collections were conducted monthly from July-September 2025 (7/10/2025, 8/7/2025, & 9/4/2025), with 60 mosquitoes collected per location per month for *kdr*-testing (total N = 360).
- Evergreen® 525 aerial treatment over Vic Fazio Wildlife Area was carried out weekly to biweekly from 7/5/2025 to 9/9/2025.



Figure 1. Locations of mosquito collection sites in the Yolo Bypass region of Sacramento, California. Conway (control site) and Vic Fazio Wildlife Area (pyrethroid-treated site).

DNA Extraction:

- Mosquitoes were dissected to remove abdomens prior to DNA extraction to minimize contamination from non-target DNA, including blood meal contents and stored sperm in the spermatheca.
- Genomic DNA was extracted from head and thorax tissues using the Qiagen DNeasy® Blood & Tissue Kit with minor protocol modifications.
- Samples were mechanically homogenized using stainless steel UFO beads to ensure efficient tissue disruption.
- To increase DNA concentration, elution was performed using 100 microliters of a 1:1 mixture of elution buffer (Buffer AE) and nuclease-free water, rather than the standard 200 microliters of Buffer AE.
- To increase DNA yield, eluted DNA was passed over the silica column twice prior to incubation in accordance with Qiagen DNeasy® Blood & Tissue Kit guidelines.

kdr Genotyping by DNA qPCR:

- Allele-specific quantitative PCR (qPCR) using SYBR Green signaling chemistry was performed with primers targeting wild-type (L) and resistance-associated (F/S) *kdr* alleles at the 1014 VGSC locus⁷.
- Reactions were performed in duplicate and included allele-specific positive and negative controls.
- Discrepant duplicate results were reprocessed.
- Genotype Identification & Validation:**
- Genotypes were initially assigned based on melt-curve analysis, with allele-specific temperature ranges used for discrimination.
- Phenylalanine (F): 76.4-76.1 °C
- Leucine (L) wild-type allele: 79.2-82.49 °C
- Serine (S): 83.1-84.2 °C



Figure 3. Melt-curve analysis showing the differentiation of *kdr* alleles. The graph plots fluorescence against temperature, showing distinct peaks for the L (wild-type) and F/S (resistance-associated) alleles.

- qPCR results with mixed leucine/serine melt peaks (L/S) were labeled ambiguous and confirmed by Sanger sequencing due to cross-amplification of a single nucleotide in codon 1014^{8,9}.
- An additional 5% of phenylalanine-homozygous samples were sequenced for confirmation as a quality control measure.

Bottle Bioassay:

- Three rounds of bottle bioassays were conducted by the Sac-Yolo MVCD to evaluate phenotypic susceptibility of *Cx. tarsalis* to pyrethrin-based adulticides on R¹⁰.
- Field collected *Cx. tarsalis* from the Vic Fazio Wildlife Area (VFWA) to pyrethrin-based adulticides.
- Known susceptible *Cx. tarsalis* reference strain (KNWR)¹⁰ as a susceptibility benchmark.
- Adult female *Cx. tarsalis* were exposed to diagnostic concentrations of pyrethrin alone and pyrethrin combined with piperonyl butoxide (PBO).
- Mortality was recorded at regular time intervals over a 180-minute exposure period.
- PBO synergistic assays were included to evaluate the potential contribution of metabolic detoxification mechanisms.

PRELIMINARY RESULTS

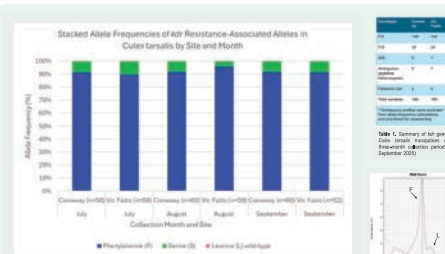


Figure 4. Stacked bar chart showing the frequency of *kdr* resistance-associated alleles (F, S, L) in *Culex tarsalis* by site and month. The chart shows that the F allele is the most prevalent across all sites and months, with some variation in the S and L alleles.

kdr Allele Frequencies (qPCR):

- A total of 360 adult female *Cx. tarsalis* mosquitoes were analyzed from each site (Conway and Vic Fazio Wildlife Area) across three collection months (July-September 2025), with 60 mosquitoes per site per month, 347 mosquitoes were confirmed out of 360 total.
- qPCR targeting the VGSC gene detected high prevalence of *kdr*-associated alleles during all collection periods at both the treated (VFWA) and untreated (Conway) sites (Table 1).
- Across all months and both sites, phenylalanine (F) was the predominant *kdr* allele (~89-96%), serine (S) occurred at lower frequencies (~4-11%), and no wild-type leucine (L/L) homozygotes were observed (Table 1).
- Despite the absence of adulticide application at the control site, phenylalanine (F) *kdr* alleles were detected at similar frequencies to the treated site (Table 1, Figure 5).
- Ambiguous melt-curve profiles, including apparent F/L peak profiles were not assigned as true F/L genotypes and were excluded from allele-frequency analyses; these samples are pending Sanger sequencing confirmation (Figure 9).
- Sequencing Confirmation:**
- Sanger sequencing confirmed the presence of the phenylalanine (F) and serine (S) alleles at the *kdr* locus.
- Sequencing of apparent F/L qPCR melt profiles were resolved as F/F genotypes, confirming the absence of L alleles (Figure 9).
- Thirteen samples remain unresolved as sequencing results were inconclusive due to limited DNA quantity, poor sequence quality, or the need for confirmatory sequencing.

Bottle Bioassay:

- Pyrethrin-only bottle bioassays of the susceptible KNWR mosquitoes exhibited rapid and complete knockdown, reaching 100% mortality by ≤30 minutes across all assay dates.
- Pyrethrin-only bottle bioassays of VFWA mosquitoes showed complete knockdown at 180 minutes (min) in July, near-complete knockdown mortality in August (one survivor), and incomplete knockdown in September (73.7% at 180 min).
- In contrast, exposure to pyrethrins formulated with piperonyl butoxide (PBO) resulted in rapid mortality, with near-complete observed as early as 30 min and complete mortality at 150 min.
- Relative to pyrethrins alone, formulations containing PBO substantially reduced knockdown time and accelerated mortality in VFWA mosquitoes.

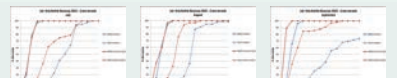


Figure 5. Bottle bioassay mortality curves for VFWA mosquitoes. The graph shows mortality percentage over time for pyrethrin alone and pyrethrin + PBO. The PBO formulation shows significantly higher and faster mortality.



Figure 6. Bottle bioassay mortality curves for VFWA mosquitoes. The graph shows mortality percentage over time for pyrethrin alone and pyrethrin + PBO. The PBO formulation shows significantly higher and faster mortality.

DISCUSSION

- Apparent F/L melt-curve profiles were not classified as true heterozygotes. Sequencing confirmed these profiles result from non-specific binding of the L-specific primer to the F-allele template, a known limitation of allele-specific qPCR⁸.
- A high prevalence of *kdr* resistance-associated alleles were detected in *Cx. tarsalis* collected from sites near Sacramento, California, with the phenylalanine (F) allele predominating serine (S) across collection months and locations^{11,12}.
- No confirmed wild-type (L/L) homozygotes were observed, consistent with resistance surveillance reports from Northern California *Cx. tarsalis* populations, where sustained insecticide selection pressure maintains resistant alleles frequency^{13,14}.
- Bottle bioassays demonstrated delayed knockdown to pyrethrin alone in VFWA mosquitoes relative to a susceptible reference strain. However, the predominance of the phenylalanine (F) allele did not result in complete resistance as mortality was still observed and not explained by *kdr* genotype alone^{15,16}.
- Bottle bioassay results demonstrated that while pyrethrins alone produced delayed mortality in VFWA mosquitoes, formulations containing piperonyl butoxide (PBO) resulted in rapid and complete mortality, which supports the involvement of metabolic detoxification mechanisms (e.g., cytochrome P450-mediated metabolism) in addition to target-site resistance^{17,18}.
- Minimal differences in *kdr* allele frequencies were observed between the control (Conway) and pyrethroid-treated (Vic Fazio WA) sites, suggesting that the high phenylalanine (F) prevalence was not solely selected during the 2025 spray season, but rather reflects pre-existing resistance allele frequencies in regional *Cx. tarsalis* populations^{19,20}.
- Molecular detection of *kdr* alleles alone does not fully predict operational control outcomes, since resistance phenotypes in *Cx. tarsalis* are multifactorial and may involve enhanced metabolic detoxification mechanisms alongside site mutations^{21,22}.
- These preliminary results highlight the importance of integrated resistance monitoring approaches that combine molecular assays with phenotypic tools such as bottle bioassays and sentinel cage trials to inform insecticide selection and resistance management strategies in mosquito control programs^{1,2,23}.
- Extended longitudinal monitoring studies would also help clarify longer-term trends or shifts in resistance profiles.

ACKNOWLEDGEMENT

- We thank the Sacramento-Yolo Mosquito and Vector Control District (Sac-Yolo MVCD) for their collaboration in this project.

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Advancing our 3D-printed trap series: The Furey 3000 Gravid Trap

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Abstract

The Marin/Sonoma Mosquito and Vector Control District (the District) annually sets over 300 gravid traps to monitor adult mosquito populations. These traps have been instrumental in detecting West Nile virus (WNV) activity, having collected more than half of the District's WNV positive mosquito pools. Unfortunately, replacing broken commercial traps can be expensive. During the 2025 adult mosquito season, the District developed and field-tested an in-house designed and 3D printed version of a gravid trap. The Furey 3000 Gravid Trap performed similarly to the commercially available option at a significantly lower cost. Using a Kestrel 1000 anemometer and field evaluations, both airflow and trap efficacy were confirmed to be comparable to the original trap. Together with the original Furey 3000 CO₂ Trap, this new gravid trap represents an innovative, practical, and cost-effective approach to adult mosquito surveillance.



Advancing our 3D-Printed Trap Series: The Furey 3000 Gravid Trap

Kelly A.L. Furey, PhD, MPH

Marin/Sonoma Mosquito and Vector Control District, Cotati, CA

ABSTRACT

The Marin/Sonoma Mosquito and Vector Control District (the District) annually deploys over 300 gravid traps to monitor adult mosquito populations. These traps have been instrumental in detecting West Nile virus (WNV) activity, having collected more than half of the District's WNV positive mosquito pools. Unfortunately, replacing broken commercial traps can be expensive.

During the 2025 adult mosquito season, the District developed and field-tested an in-house designed and 3D printed version of a gravid trap. The Furey 3000 Gravid Trap performed similarly to the commercially available option at a significantly lower cost. Using a Kestrel 1000 anemometer and field evaluations, both airflow and trap efficacy were confirmed to be comparable to the original trap. Together with the original Furey 3000 CO₂ Trap, this new gravid trap represents an innovative, practical, and cost-effective approach to adult mosquito surveillance.

METHODS

TESTING TRAP EFFICACY

- Traps set ~ 20 feet from each other on same night
- Traps set in same location on consecutive nights

METHODS

TRAP PRINTING

Design Program	Rhinoceros 8.0
Filament	Creality PETG Filament 1.75
Nozzle Size	0.4 mm

"Maximus"

Bambu Lab X1 Carbon



- 256x256x256 build volume
- Textured bed
- Max speed up to 500mm/s

"Fufu"

Creality K2 Plus



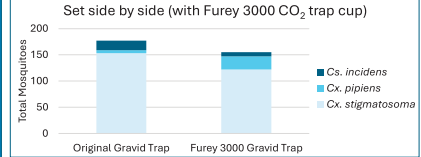
- 350x350x350 build volume
- Textured bed
- Max speed up to 600mm/s

FIELD TRIALS

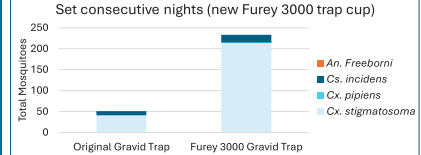


RESULTS

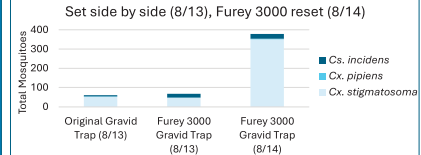
Week 31



Week 32



Week 33



DISCUSSION

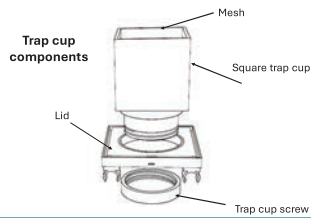
- The Furey 3000 Gravid Trap performs comparably to commercial traps at a substantially lower cost
- Standardizing the fans and batteries across trap designs simplifies maintenance and part replacement
- When possible, reusing 3D-printed components across trap types reduces production time and overall costs

ACKNOWLEDGEMENTS

This product would not be what it is without the helpful discussions and field testing of many individuals, including E. Lloyd Furey, Rob McGovern, Casey Richter, Nizza Sequeira, Peter Bonkrude, Nathaniel Reed, Kristen Holt, Sarah Brooks, Elijah Alter, Steven Woodall, and Hannah Kiehl.

METHODS

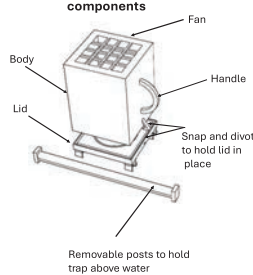
TRAP COMPONENTS



NOTE: During the initial testing phase (week 31), the trap cup from the Furey 3000 CO₂ trap cup was used. However, its limited surface area was insufficient to accommodate the number of mosquitoes collected when inverted. The revised trap cup design above improved collection capacity in subsequent weeks.



Main trap body components



Removable posts to hold trap above water

The Hotswap Trap: A modular, low-cost EVS trap with minimal fabrication and easy maintenance

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Introduction

Rising trap costs and recurring replacement of malfunction-prone parts can absorb an increasing share of limited surveillance resources. Field-serviceable designs that use both durable core components and easily replaceable wear-prone parts may reduce trap failures, downtime for trap availability and limit the accumulation of non-functioning equipment. In addition, using an in-house scent attractant reduces the annual cost of purchasing lures for all field trap designs.

Methods

The Hotswap dry-ice-baited EVS trap incorporates prefabricated components with minimal modification, reducing assembly time. The trap consists of an ABS coupling, 4 in. × 4 in. (Grainger, Lake Forest, IL), fitted with a PVC S&D reducer coupling, 3 in. Hub × 4 in. Hub (National Diversified Sales, Woodland Hills, CA) secured by a custom metal rod hanger, with a 12 V duct fan (Guangzhou Tongya Electronic Commerce Co., Guangzhou City, Guangdong) mounted at the opposite end and powered by a USB-A connection (Shenzhen HiLetgo Technology Co., Shenzhen, Guangdong) to a 5 V power bank (Hong Kong Kemaiqiang Trading Limited, Fanling, Hong Kong). A vinyl phone pouch (Shenzhen FulTV Trading Co., Shenzhen City, Guangdong) mounted to the side of the trap holds the power bank and isolates rust-prone electronic components from condensation accumulating around the dry-ice bag. The scent attractant was produced by loading dilute lactic acid (Mathew et al. 2013) into a carrier medium of crystal litter and silica beads.

Results and Discussion

The total assembly time for both the dry-ice bag and trap was 6.5 minutes each. The dry-ice bag held 2.77 kg of dry

ice, and intake wind speed measured with a Kestrel 5500 weather meter (Boothwyn, PA) was 4.6 m/s. At the default power bank output of 5.068 V, the trap drew 274 mA and operated for 52 hours when powered by a commercial power bank rated at 40,000 mAh. The previously used trap cost \$237.30 per unit, whereas the Hotswap trap cost \$55.21 per unit. Replacement costs for wear-prone components, including the motor, corroded USB connector, and scent-depleted lure, were \$90.60 for the previous trap and \$23.53 for the Hotswap trap not including labor.

Conclusion

Because this was the first year of deployment, time-to-failure and repair frequency under routine field handling and weather exposure have yet been to be quantified. A notable advantage from a typical CDC light trap baited with CO₂ (Centers for Disease Control and Prevention 2024) was the omission of a light attractant and other insect by-catch. Compared with the trap previously purchased from an outside vendor, the in-house Hotswap trap reduced unit cost by 76.73% and replacement-part cost by 74.03%.

Acknowledgements

We thank the staff of the Alameda County Mosquito Abatement District for their recommendations.

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Fire in the sand: Efforts to control Red Imported Fire Ants in the desert

Victor Scrima, Robert Mora*, Antonio Molina, and Gregorio Alvarado

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Introduction

The Red Imported Fire Ant (RIFA), *Solenopsis invicta*, is an invasive species that has become well established in the Coachella Valley since its initial detection in 1998. Historically, infestations were primarily associated with irrigated turf and grassy landscapes, allowing for clearly defined inspections and treatment boundaries. In recent years, however, RIFA populations have demonstrated notable adaptation to arid desert environments. Colonies are increasingly being detected in decomposed granite (DG), sand, and other xeriscape settings, often with limited water sources (Figure 1). This ecological shift presents new challenges for detection and control due to reductions in visibility and expanded habitat range. The Coachella Valley Mosquito and Vector Control District (CVMVCD) remains committed to mitigating the spread of RIFA through adaptive and proactive control strategies.

Methods

To address these emerging challenges, CVMVCD has implemented and evaluated modified inspection and treatment protocols tailored to desert environments. Standard surveillance methods remain in place within turf and irrigated areas, including confirmation of RIFA presence and application of Extinguish® Plus fire ant bait. Field

applications are conducted using backpack sprayers and targeted baiting techniques. Additional strategies are being tested in desert landscaping, including broad-area treatments in high-risk zones and inspection strategies in low-visibility environments. Technicians conduct detailed inspections to locate colonies in non-traditional habitats where mound structures are less visible (Figure 2). These approaches aim to reduce non-target impacts on native ant species, which are prevalent in desert ecosystems, while improving overall treatment effectiveness.

Results and Discussion

The Coachella Valley encompasses multiple municipalities, including Cathedral City, Coachella, Desert Hot Springs, Indian Wells, Indio, La Quinta, Palm Desert, Palm Springs and Rancho Mirage (Figure 3). In 2024, CVMVCD conducted inspections at 1,125 residential properties, and in 2025 that number increased to 1,325 properties. (Figure 4). This increase reflects a rise in service requests and expanded surveillance efforts across the region. This trend also may be attributed to RIFA's continued adaptation to desert landscapes and regional water conservation efforts that promote xeriscaping. Rather than declining, RIFA populations have been expanding into dry environments such as decomposed granite and sand. Field



Figure 1.—RIFA mound at base of rock.



Figure 2.—Technician inspecting RIFA.

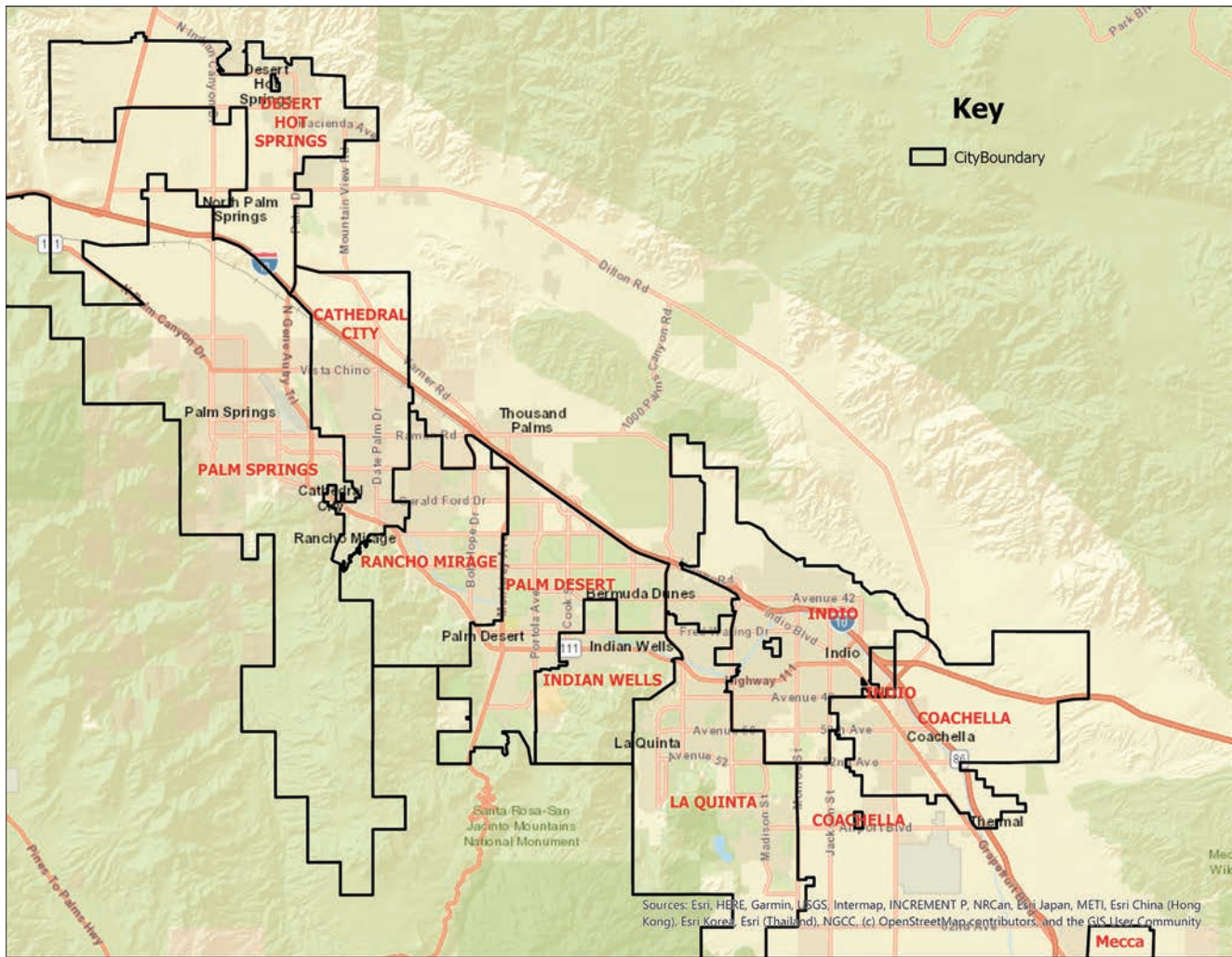


Figure 3.—Map of the Coachella Valley.

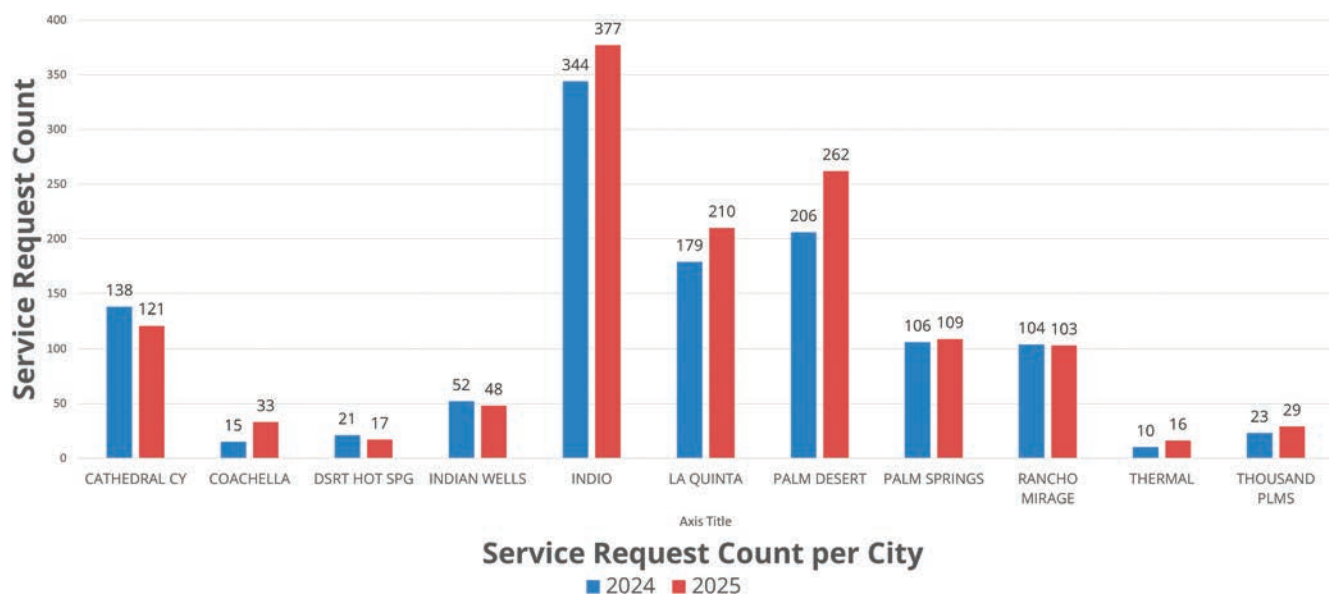


Figure 4.—Service request count per city.



Figure 5.—RIFA mound at the base of tree in desert landscaping.

observations indicate that colonies are establishing farther from irrigation sources and into areas previously considered uninhabitable (Figures 5 and 6). CVMVCD's management approach has evolved to incorporate adaptive strategies that address these behavioral changes in RIFA while remaining effective and efficient. Public outreach and education efforts also have been emphasized to improve early detection and reporting, enabling rapid response and treatment (Figure 7).

Conclusion

Effective management of RIFA in desert environments requires a combination of proactive monitoring, adaptive treatment strategies and community engagement. Key components include early detection, strategic application of control products, and consideration of non-target species in desert ecosystems. Public outreach efforts further strengthen



Figure 6.—RIFA mound at the base of a large boulder in desert landscaping.



Figure 7.—Technician educating residents and providing a RIFA brochure.

control efforts by encouraging community participation and improving response times. Through integrated vector management practices and continued collaboration with residents, CVMVCD aims to minimize the impact of RIFA on public health, agriculture and the environment.

Acknowledgements

The District would like to express its gratitude to the Laboratory, Operations, and Public Outreach department staff for their valuable feedback and editorial support. Additionally, we extend a special thanks to the Red Imported Fire Ant crew for their field assistance and their continued dedication to managing invasive species and promoting public health.



Fire in the Sand: Efforts to Control Red Imported Fire Ants in the Desert

Victor Scrima, Vector Control Technician II; Robert Mora, Field Supervisor; Antonio Molina, Operations Program Coordinator; Gregorio Alvarado, Operations Manager

ABSTRACT: The Red Imported Fire Ant (RIFA), *Solenopsis invicta*, has become an invasive species in the Coachella Valley, adapting from grassy environments to desert landscaping. This shift poses new challenges for control efforts, as RIFA colonies are now spreading over larger areas and forming smaller, more compact mounds that are harder to detect and treat. Additionally, RIFA's ability to thrive in drier conditions complicates management strategies. In response to these challenges, the Coachella Valley Mosquito and Vector Control District (District) is committed to proactive monitoring, innovative treatment methods, and public education to limit the spread of this invasive pest and protect community spaces. The District aims to keep RIFA populations at levels that do not create nuisances or public health concerns. In this poster, we will highlight the significance of effective communication between residents and the District, as well as the selection of appropriate control products and application equipment to manage RIFA populations.

INTRODUCTION: The Red Imported Fire Ant (RIFA), *Solenopsis invicta*, is an invasive species that has become established in the Coachella Valley. Traditionally associated with irrigated and grassy environments, RIFA have adapted to desert landscaping and arid conditions. This adaptation presents new challenges for detection and control as colonies are often harder to detect. The District is committed to stopping the infestation of Coachella Valley from this pest and preventing the migration of this species to other areas.



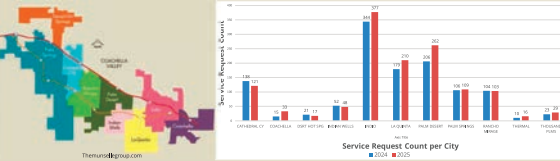
OBJECTIVE: Raise awareness and decrease the populations of Red Imported Fire Ants (RIFA).

- Describe the challenges of managing RIFA in desert environments.
- Emphasize the role of public communication in detection.

METHODS: Broadcast treatments of Extinguish Plus® Insecticide Fire Ant Bait (active ingredients: 0.365% hydramethylnon and 0.250% S-Methoprene) were made to residential properties using a hand spreader or Maruyama at 1.5 lbs. per acre.



OVERVIEW: The Coachella Valley includes the cities of Cathedral City, Coachella, Desert Hot Springs, Indian Wells, Indio, La Quinta, Palm Desert, Palm Springs, and Rancho Mirage. In 2024, we inspected 1,125 residential properties and 1,325 additional properties in 2025, reflecting an increase of service requests.



The rise in service requests may be attributed to the RIFA adapting to desert landscapes and due to new developments in the Coachella Valley. For example, RIFA mounds are now being discovered around barrel cactuses, drip irrigation systems, and in drier areas where they were historically not present. Over the past few years, RIFA have been finding suitable moisture in these desert habitats. The District's practical management of RIFAs in desert habitats relies on adaptive strategies that account for changes in colony behavior and reduced visibility. Educating our community on how to identify RIFA on their properties will enable the District to respond rapidly to control this invasive species and reduce the threat to residents, beneficial insects, and crops.



CONCLUSION: Key components of successful control include proactive monitoring, hotdog baiting careful selection of appropriate products, and specialized application methods. Additionally, strong communication between residents, community, and the District is vital, as it facilitates early detection and prompt responses. By implementing integrated management practices and engaging in public outreach, RIFA technicians can maintain population levels that minimize nuisance and public health issues.



ACKNOWLEDGEMENTS: The District would like to express its gratitude to the Laboratory, Operations, and Public Outreach department staff for their valuable feedback and editorial support. Additionally, we extend a special thanks to the Red Imported Fire Ant crew for their field assistance and their continued dedication to managing invasive species and promoting public health.

For further information, contact Victor Scrima at the Coachella Valley Mosquito and Vector Control District at VScrima@cvmosquito.org

From service request to prevention: A community-driven mosquito control model in the Coachella Valley

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Introduction

Mosquito-borne disease risk is elevated in regions with favorable climates and abundant urban larval habitats, such as the Coachella Valley. Although resident service requests often provide an early indication of mosquito activity, they are frequently underutilized in proactive control strategies. The current project demonstrates how structured response protocols and community engagement can transform service requests into an effective surveillance and prevention tool.

Methods

A five-step workflow beginning with completion of a community service request intake form (Fig. 1) is followed by field inspection and surveillance to identify active and potential larval sources. Technicians conduct property and adjacent area inspections, and document findings using digital tools (Fig. 2). Integrated Vector Management (IVM) strategies, including physical source reduction, larvicides/adulticides, and biological control such as *Gambusia affinis*, are applied as appropriate. Education and outreach efforts support long-term prevention and resident engagement (Fig. 3).

Results and Discussions

The structured workflow improved early detection of cryptic and overlooked mosquito sources not identified during routine inspections, including neglected containers, storm drains, and hidden water features

(Fig. 4). Use of digital tools enhanced data accuracy, localization, and decision-making for targeted interventions. Community engagement increased awareness and encouraged preventative behaviors, contributing to a reduction in repeated service requests over time. These findings highlighted the effectiveness of combining public participation with systematic surveillance and IVM practices, aligning with broader public health goals of reducing mosquito populations and disease risk.

Conclusions

Community service requests can serve as a valuable proactive surveillance tool when paired with structured response protocols and integrated control strategies. This approach enhances early detection, reduces larval sources, improves operational efficiency, and supports sustainable mosquito management and public health protection.

Acknowledgements

I thank the Operations, Laboratory, and Public Outreach departments of the Coachella Valley Mosquito and Vector Control District for their contributions and support. Special thanks are extended to the field technicians for their continued dedication to mosquito control and public health. I would also like to thank the MVCAC, General Manager Jeremy Wittie, Operations Coordinator Antonio Molina, and my Field Supervisor Jeff Rushing for the opportunity to present this poster.



Coachella Valley Mosquito & Vector Control District

REQUEST A SERVICE
(/REQUEST-A-SERVICE)

Request a Service

To request a service from the District, you may either:

- call the District Call Center at (760) 342-8287
 - Press 1 for Red Imported Fire Ants (RIFA)
 - Press 2 for Mosquitoes and all other calls
- complete the fields below which will be sent to the District Call Center

For RIFA service requests (including city parks, HOA common areas, schools, golf courses, businesses, etc.) please fill out the form here. (<https://cvmvcd.specialdistrict.org/requesting-red-imported-fire-ant-rifa-treatment-for-large-or-commercial-properties>)

A Call Center representative will contact you within the next 72 business hours to complete your service request.

Name*

Company Name

Street*

Zip*

City*

Building Number

Apartment / Suite Number

Access*

Phone Number*

Email*

Service Request Type*

How did you hear about us?*

Comments or Questions

<https://www.cvmosquito.org/request-a-service>

Figure 1.—Community engagement and service request submission form.

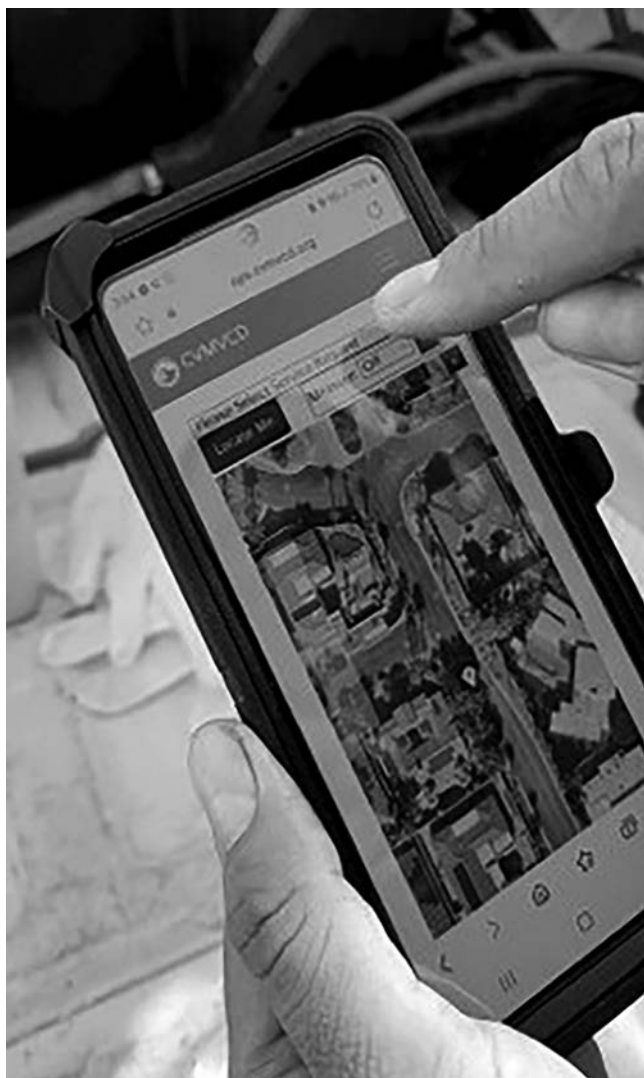


Figure 2.—Digital data collection and mapping tools used by field technicians.



Figure 3.—Education and prevention strategies to reduce mosquito sources and increase awareness.



Figure 4.—Field inspection and identification of mosquito breeding sources.



From Service Request to Prevention: A Community Driven Mosquito Control Model in the Coachella Valley

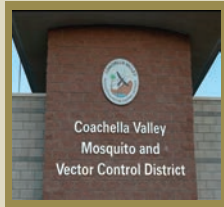
Christopher Vazquez, Vector Control Technician I; Jeffrey Rushing, Field Supervisor;
Antonio Molina, Operations Program Coordinator; and Gregorio Alvarado, Operations Manager

Public Health Challenge:

The Coachella Valley's warm climate and urban landscape create ideal, year-round breeding conditions for invasive and endemic mosquito species, increasing the risk of mosquito-borne disease transmission. Community service requests often represent the first indicator of localized mosquito activity, yet without structured follow-up, these reports can remain reactive rather than preventative.

Program Overview:

The Coachella Valley Mosquito and Vector Control District uses service requests submitted by residents as a proactive surveillance and prevention tool. Each request initiates a structured response that includes field inspection, source identification, target control measures, and resident education. Inspection and treatment data are recorded digitally to support data-driven decision-making and long-term prevention strategies.



Step 1: Community Engagement and Service Requests

- Submissions from residents drive field response
- Requests are scheduled
- Community participation promotes prevention



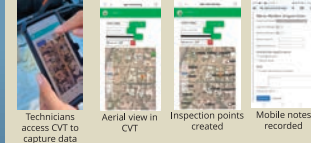
Step 2: Field Inspection and Surveillance

- On-site property inspection
- Identification of active and potential breeding sources
- Expanded inspections of surrounding area (neighboring properties, storm drains, retention basins, hidden habitats)



Step 3: Data Collection and Decision-Making

- Digital field tools (CVT)
- Mobile notes, photos, and treatment records
- Data supports targeted, evidence-based control actions



Informed residents → fewer breeding sources → reduced future service requests

Step 4: Integrated Control Procedures

- Integrated Vector Management (IVM)
- Physical source reduction
- Larvicides and adulticides (as appropriate)
- Biological means when practical



Step 5: Education and Prevention

- Resident education on source reduction
- Personal protection guidance
- Risk communication and empowerment



Material and Equipment:

- Solo/Stihl sprayer systems
- EPA Registered larvicides/adulticides
- PPE for safe applications
- Mobile data-entry platforms



Public Health Results:

- Reduce mosquito breeding through early detection
- Increase public awareness and engagement
- Lower risk of mosquito-borne disease transmission
- Transition from reactive response to proactive prevention
- Healthier, better-informed communities



Acknowledgement:

The District would like to express its gratitude to the Operations, Laboratory, and Public Outreach department staff for their valuable feedback and editorial support. Additionally, we extend a special thanks to the urban/rural Mosquito crew for their field assistance and their continued dedication to managing invasive species and promoting public health.

For further information, contact Christopher Vazquez at the Coachella Valley Mosquito and Vector Control District at cvazquez@cvmosquito.org

Case study of a kissing bug infestation (*Hospesneotomae protracta*) in a residential home in Orange County, California, 2018

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*Corresponding author: lkrueger@ocvector.org

Introduction

The Orange County Mosquito and Vector Control District (OCMVCD) insect identification program receives between one and three adult *Hospesneotomae protracta* (Uhler 1894),

the western conenose bug, annually. In September 2018, a resident submitted photos of adult and nymphal kissing bugs found in the bed and bedroom for identification. This is the only confirmed occurrence of *H. protracta* nymphs and adult life stages collected from inside a



Figure 1.—Adult *H. protracta* bug removed from bed and flushed down toilet (inset image is magnified adult).

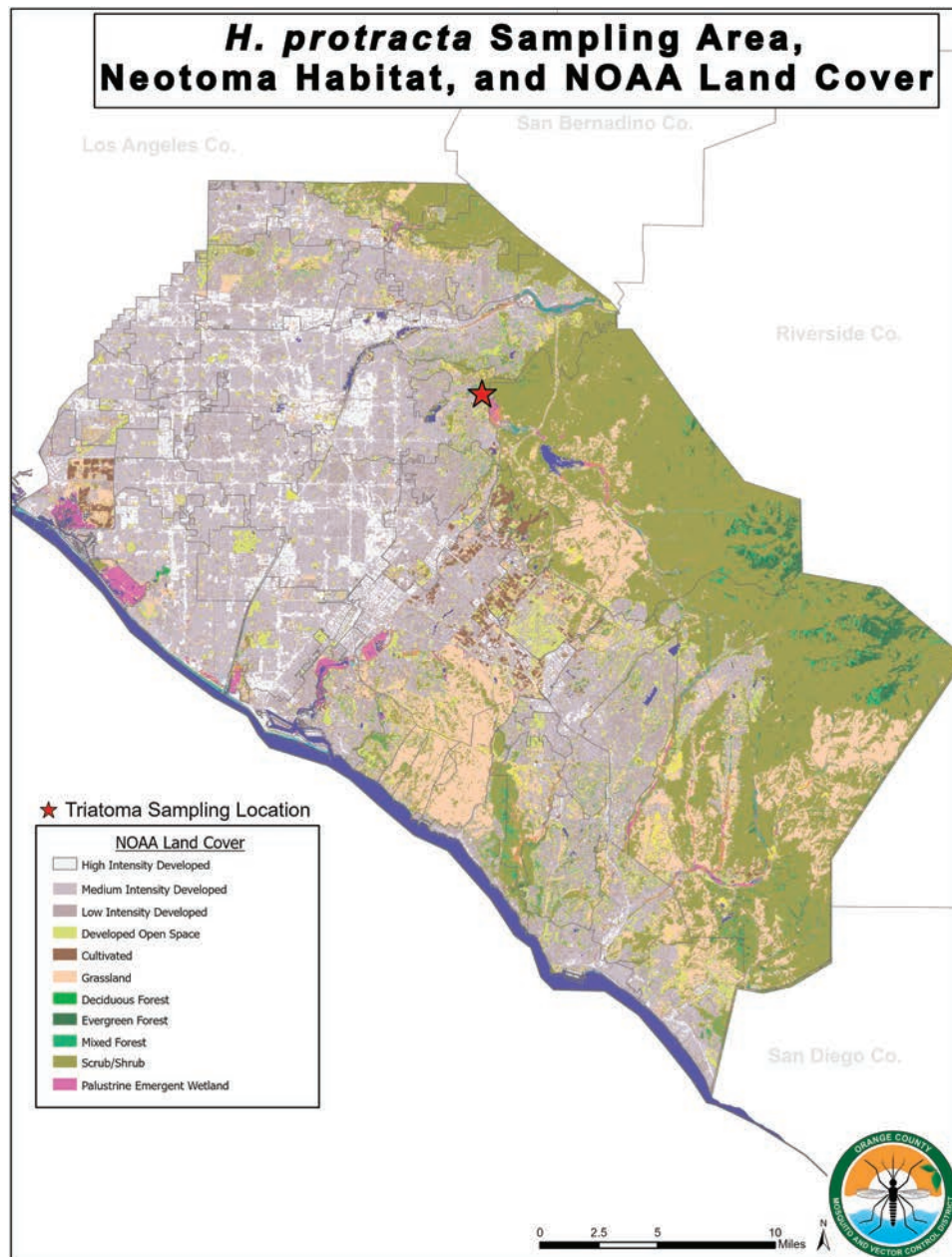


Figure 2.—*H. protracta* sampling area, *Neotoma* habitat, and NOAA Land Cover, Orange County, CA.

home in Orange County and submitted to the OCMVCD insect identification program. This case report describes a 2018 infestation of kissing bugs occurring inside a residence in Orange County and the recommendations provided to the resident to prevent kissing bugs from entering the home.

Hospesneotomae protracta is a kissing bug (Hemiptera: Reduviidae) associated primarily with the nests of big-eared (*Neotoma macrotis*) and dusky-footed (*N. lepida*) wood rats and is an obligate blood feeding ecto-parasite considered an occasional invader of homes in Orange County. Woodrats are common in oak woodland, chaparral, and coastal sage scrub habitats and adjacent communities in the wildland urban interface.

Case Study of Infestation, 2018

In September 2018, a resident in the City of Orange contacted OCMVCD requesting identification of bugs found in her bed (Figure 1). Unable to confirm the species from the photos, but suspecting *H. protracta*, OCMVCD scheduled an inspection of the property. On 17 Sep OCMVCD Vector Ecologist Krueger inspected the exterior of the home and found signs of wood rat activity and abundant wood rat habitat adjacent to the development (Figure 2). The resident lived in a large single family residential home within a Homeowners Association adjacent to open space (Figure 3). The resident reported she found the first bug on 13 Aug when she felt it crawling on her in her bed. She squished the



Figure 3.—View from French doors of upstairs bedroom where *H. protracta* nymphs were collected.

bug, noticed blood, and took a photo before flushing it down the toilet (Figure 1). The next day, the resident noted a large bug bite on her leg. A few weeks later 5 Sep, after noticing bites on her body, the resident discovered two nymphs (Figure 4) near the bed.

Feeling concerned, the resident contacted OCMVCD requesting identification of the bugs using the photos. The resident requested an interior inspection where the following conditions were noted: French doors open and leading to balcony with no screens, exterior lighting on exterior of home near French doors, bed on ground to facilitate co-sleeping with toddlers, and presence of a dog that frequently roamed the exterior patio. Upon closer inspection of the bed, Vector Ecologist Krueger collected ten blood-fed nymphs between the mattress cover and mattress pad and along seams (Figure 5 & Figure 6). The homeowner was provided with

recommendations listed in Table 1 and sought medical consultation. At that time, the physician did not recommend testing for *Trypanosoma cruzi*, the etiologic agent of American trypanosomiasis, also known as Chagas Disease.

Public Health Risk from Kissing Bug Bites

Over 1.3 million residents of Orange county live within the home range of woodrats (Figure 2). Research shows 7% of people are hypersensitive to kissing bug saliva (Marshall 1986). Repeated exposure to the saliva increases the risk for anaphylaxis reactions from bites. From 2008-2024 OCMVCD received < 3 reports/year from residents of bite reactions to bugs. In 2025, a year with increased media awareness of kissing bug bites and American trypanosomiasis, OCMVCD received



Figure 4.—Two dead *H. protracta* nymphs photographed by Orange County resident (inset image is magnified nymph).

14 reports of adult *H. protracta* bug bites, with two residents reporting an allergic reaction.

Because the overall risk of a kissing bug bite to the general Orange County public is low, the risk of acquiring American trypanosomiasis is also low. Studies show the prevalence of *T. cruzi* can be as high as 33% in kissing bug populations in Southern California (Sjogren 1965, Wood 1975). However, only one human case of Chagas disease has been reported from Orange County,

with the exposure listed as unknown (VBDS 2024). The overall risk of acquiring American trypanosomiasis in Orange County is considered low due to infrequent human kissing bug contact and the limited vectorial competence of *H. protracta*. This case study highlights that although the risk of kissing bug bites is low, it is not zero, and kissing bugs can cause infestations inside residences in Orange County adjacent to wood rat habitat.



Figure 5.—Image of *H. protracta* blood fed nymphs collected from bed, photo by OCMVCD.



Figure 6.—Collection location of ten *H. protracta* nymphs between mattress and pad of bed, photo by OCMVCD.

Table 1.—Recommendations to Prevent Kissing Bugs from Entering and Infesting Residential Homes (UT Health 2018).

Recommendation	Action Item	Specific Mitigation Steps
Structural Sealing	Exclusion	Maintain screens; seal cracks and gaps; install door sweeps, close chimney flues
Outdoor Management	Habitat Removal	Remove firewood, lumber and brush piles from around foundation.
Rodent Control	Vector Reduction	Remove rodent nests within 300 feet from residences, trim vegetation overhanging the structure.
Lighting	Reduce Attraction	Use yellow “bug lights” or turn lights off; move light fixtures away from doors/windows, replace interior lighting with yellow “bug lights”, turn off swimming pool lighting before dusk and leave off until after dawn.
Indoor Precautions	Bedding & Pets	Wash bedding regularly; move beds away from walls, inspect bed regularly during peak <i>H. protracta</i> activity (July-September)
Pet Health	Pets & Parasite Control	Bring pets inside at night; keep pets on regular flea and tick treatments, regularly wash pet bedding, inspect pets after returning from being outdoors for bugs.
Chicken Coop Management	Pets & Parasite Control	Regularly inspect chickens and coops for <i>H. protracta</i> ; screen coops to prevent bug entry and establishment.

Acknowledgements

Thank you to the OCMVCD laboratory, Brian Brannon, and the OC resident who shared the photos of kissing bugs and granted permission to publish.

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Detection of helminth parasites *Mastophorous muris* and *Taenia taeniaeformis* in roof rats (*Rattus rattus*) collected from residential backyards in Orange County, California, 2023-2024

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Abstract

The Orange County Mosquito and Vector Control District (OCMVCD) conducts surveillance of roof rats (*Rattus rattus*) in residential areas of Orange County to identify and respond to potential public health threats. In 2024, OCMVCD identified the presence of two helminth parasites, *Taenia taeniaeformis* (cat tapeworm) and *Mastophorus muris* (a globally distributed parasitic nematode of rodents) in roof rats that were collected in residential backyards in Tustin and Irvine. A rat collected by University of California Agricultural and Natural Resource staff in a residential backyard presented with helminths encysted in the liver during necropsy. In Tustin roof rats (6 positive detections as of November 2025) were collected in lethal traps in response to reports of high rodent, squirrel, and cat activity at a large Home Owners Association (HOA). The worms were observed to exit the anus and mouth upon collection by lethal traps. To identify these rat-borne helminthiases, DNA was extracted from representative specimens collected from both locations. The 18S and 28S rRNA genes were sequenced using 18S and 28S rDNA primer sets for next-generation sequencing (NGS)-based parasite detection. *Taenia taeniaeformis* found in roof rats presents a risk to domestic cats, which become infected by consuming infected rats. Although rare, humans can be infected by *T. taeniaeformis*. *Mastophorus muris* is a parasite of rodents and not considered to be of public health importance. This poster describes the methods used to speciate internal helminth parasites of roof rats and provides recommendations for Orange County residents concerned with roof rat infestations on their property.

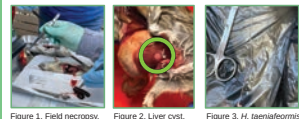
Detection of Helminth Parasites (*Hydatigera taeniaeformis* and *Mastophorus muris*) Collected From Roof Rats (*Rattus rattus*) in Orange County, California, 2023-2024

Laura Krueger¹, Daisy Flores-Rangel¹, Niamh Quinn², Dave Taylor¹, Amber Semrow¹
¹Orange County Mosquito and Vector Control District, Orange County, CA
²UC ANR, South Coast Research Center, Irvine, CA

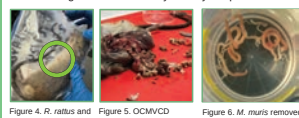


Introduction

Roof rats (*Rattus rattus* Linnaeus 1758) are present in all areas of Orange County. The Orange County Mosquito and Vector Control District (OCMVCD) conducts a rodent surveillance program whereby rodents are tested for infectious agents and examined for ectoparasites. From 2008-2024, no rats showed obvious indications of helminth infection. In 2023, OCMVCD received a report of a roof rat infested with an unknown helminth from Dr. Quinn, University of California Agriculture and Natural Resources (Figure 1, 2, 3). To establish if the helminth was a public health threat, OCMVCD agreed to molecularly identify the parasite.



OCMVCD staff trapped roof rats at a Tustin, Calif., homeowners association (HOA) in September 2024 due to complaints of animal feeding and high abundance. Worms were observed exiting the mouth of a deceased roof rat (Figure 4). During necropsy helminths were observed in 6 of 9 roof rat carcasses from the HOA (Figure 5). According to detailed records kept since 1986, this was the first time OCMVCD observed a helminth exiting a rodent carcass. To establish if the helminth was a public health threat, OCMVCD agreed to molecularly identify the parasite.



Methods: Trapping

Roof rats were collected in lethal snap traps placed on residential properties (*H. taeniaeformis*) by UCANR and common areas of an HOA (*M. muris*) by OCMVCD. UCANR performed a field necropsy and OCMVCD necropsied roof rat carcasses in the laboratory.

Methods: Sequencing & Speciation

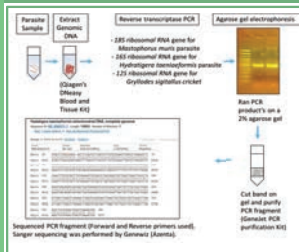


Figure 7. Approximately 1 cm of the parasite was used to extract genomic DNA using the Qiagen's Blood and Tissue Kit and the following primers were used for species identification. For *Mastophorus muris*, the 18S forward: 5'-GCCAGCAVCYCGGTAAY-3' and reverse: 5'-CCGTCAATHTCTTYAART-3'. For *Hydatigera taeniaeformis*, the 12S forward: 5'-TCGTGCGGACGCTCAGATGTGTATAAGACAGCGTGYDAAGGTAGSATAAT-3' and reverse: 5'-GTCTCGTGGGCTCGGAGATGTGTATAGACAGCCGGTYTYAACTCARTCAT-3'. The 12S primer set used to detect the presence of *Gryllobates sigillatus*, forward: 5'-TCGTGCGGACGCTCAGATGTGTATAAGACAGCGTGCAGCATCYCGGTTA-3' and reverse: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGACAGCGGTGACGGCGGTGTAC-3'.

PCR conditions: 94°C for 2 min, followed by 94°C for 30 sec, forty cycles of 52°C or 62°C for 45 sec and 72°C for 1 min, with a final extension step of 72°C for 5 min, gel band of the appropriate size was cut out of the gel and purified using the GeneJet PCR purification kit. Once the PCR amplicon was purified it was sent to Genewiz (Azenta) for Sanger sequencing. Sequence FASTA result was analyzed using the BLAST (National Center for Biotechnology Information) and species was revealed. Sequences were analyzed in the forward and reverse reactions separately and similar results were obtained to confirm the species identification.

Hydatigera taeniaeformis Results & Discussion

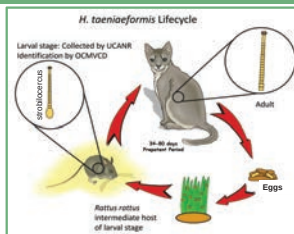


Figure 8. Lifecycle of *H. taeniaeformis*. Image modified from NC State University.

H. taeniaeformis (Batch, 1786), the cat tapeworm, is a parasitic helminth with an indirect lifecycle that requires two different hosts to complete development. Previous names include *Taenia taeniaeformis* and *Strobilocercus fasciolaris* (larval stage). The tapeworm was removed from a cyst in a field necropsied *Rattus rattus* by UCANR staff. The adult stage lives in the small intestine of felids but can also be found in dogs and humans.

Rattus rattus is an intermediate host infected by consuming cat feces with eggs. The eggs develop into a strobilocercus and encyst within liver tissue (Figure 2). The cyst provides a physical barrier that shields the larvae from the host's immune system, ensuring they remain viable. Once ingested by a cat, the cyst is digested, allowing the tapeworm to attach to the intestinal wall and mature into its adult form (Figure 8). *H. taeniaeformis* infection in humans is rare due to the fecal-oral transmission route.

Prevention Recommendations

To prevent *H. taeniaeformis*:

- Control rodent intermediate hosts
- Routine veterinary care including deworming of pets
- Ensure that cat food and water are stored in areas inaccessible to wild rodents. Avoid feeding cats raw fish or rodents.
- Wash hands after handling cats, cleaning litter boxes, and outdoor activities.

Mastophorus muris Results & Discussion

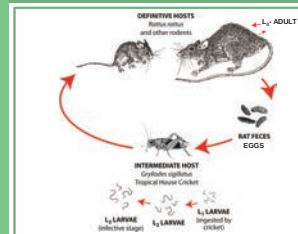


Figure 9. Lifecycle of *M. muris*.

Mastophorus muris (Gemin 1794) is a parasitic nematode (roundworm) that lives in the stomach of rodents with an indirect life cycle that requires an intermediate host (Figure 9). This study reports the first confirmed detection of *M. muris* in *R. rattus* in California and the first confirmed detection of *Gryllobates sigillatus* in Orange County (Figure 9). The cycle begins when a suitable intermediate host (detected by OCMVCD as *Gryllobates sigillatus*, the tropical house cricket) ingests *M. muris* eggs passed in the feces of an infected rodent. Inside the insect, the larvae develop into an infective stage. Rats become infected by eating the infected intermediate host. Once ingested, the larvae are released, penetrate the stomach wall, and develop into mature adult worms. Adult worms mate in the stomach of the rodent, and female worms produce eggs, which are then passed out in the host's feces, contaminating the environment to start the cycle again. The *M. muris* parasite does not infect humans.

Gryllobates sigillatus, the tropical house cricket, is native to Asia but has spread worldwide due to the commercial production of the crickets for feeding pets like reptiles, birds, amphibians, and insectivorous arthropods.

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- Kounosu, A. (2019) *Scientific Reports*. Improved 18S and 28S rDNA primer sets for NGS-based parasite detection.

Excelling beyond Excel

Melissa Snelling* and Kim Y. Hung

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Abstract

In 2025, the Coachella Valley Mosquito and Vector Control District began using Tableau (Salesforce Inc., Seattle, WA), a business intelligence and data visualization software, to examine trends in mosquito surveillance data by city and trap, track mosquito pool virus results, compare site productivity, assess species prevalence across areas of interest, and evaluate field trials on a spatial view. Although there is much still to learn about using this tool, strides have been taken to incorporate daily data analytics in Tableau across the District. This poster presents a few of the dashboards that have been created over the past year.



Excelling Beyond Excel®

Using Tableau® to Visualize Mosquito Surveillance Data

Melissa Snelling and Kim Y. Hung, Ph.D., BCE
Coachella Valley Mosquito and Vector Control District

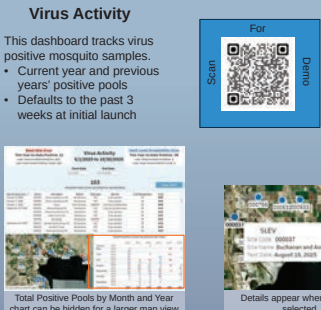


Many of us work with endless spreadsheets and a sea of numbers that inform our decisions and drive our next steps. Tableau is a data analytics platform that transforms those spreadsheets and databases into graphic visualizations that are interactive and easier to understand. As it is said, a picture is worth a thousand words, right? Tableau engages the user by allowing them to interact with visualizations, which go beyond static line graphs and bar charts, so they can find answers to their questions. In 2025, the Coachella Valley Mosquito and Vector Control District began using Tableau to examine trends in mosquito surveillance data by city and trap; track mosquito pool virus results; compare site productivity, assess species prevalence across areas of interest; and evaluate field trials on a spatial view. While there is much still to learn about using this tool, strides have been taken to incorporate Tableau into daily data analytics across the District.

Virus Activity

This dashboard tracks virus positive mosquito samples.

- Current year and previous years' positive pools
- Defaults to the past 3 weeks at initial launch



Total Positive Pools by Month and Year chart can be hidden for a larger map view

Larvicide Treatments

An all-in-one view of the missions by selected date of treatment.

- Truck route
- Time of application
- Larval test cup locations
- Adult mosquito trap locations
- Weather chart with time, wind speed and direction, and temperature in a single view

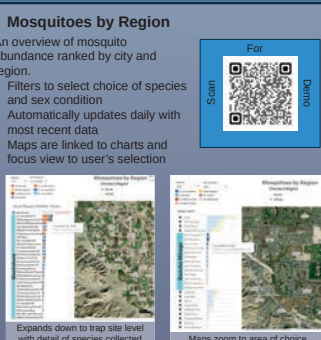


Embedded details within the map appear with hover action

Mosquitoes by Region

An overview of mosquito abundance ranked by city and region.

- Filters to select choice of species and sex condition
- Automatically updates daily with most recent data
- Maps are linked to charts and focus view to user's selection

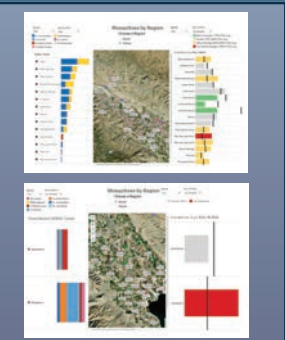


Expands down to trap site level with detail of species collected

Mosquito Dispersal

This dashboard illustrates the results of a mosquito mark release recapture trial done as part of a Sterilized Insect Technique study.

- Center is the release point
- 20 trap sites set daily around release point
- View changes dynamically to match the filter choices



Buffer distance and date are changeable

For further information, please contact Melissa Snelling at msnelling@cvmosquito.org

Special Thanks to Andy Kriebel at Next Level Tableau training for the instruction, inspiration and guidance
www.nextleveltableau.com

Comparing efficiency of glass feeding apparatus and Hemotek feeding system for *Aedes aegypti* colony maintenance

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Abstract

The Sterile Insect Technique (SIT) relies on maintaining healthy mosquito colonies in insectaries, which in turn depends on efficient blood-feeding methods to ensure consistent egg production—particularly for mass rearing and sustained sterile male releases. Optimizing feeding procedures not only supports colony health but also enhances operational efficiency and resource utilization. Our study compared the performance of two mosquito blood-feeding apparatuses—the Hemotek feeding system and the glass membrane feeder—to evaluate their relative efficiency in supporting *Aedes aegypti* colony maintenance. Three cages were set up for each feeding method, each cage containing 100 males and 100 females. Feeding success rates and subsequent egg production were evaluated and analyzed to assess performance. Results showed that 88.6% of female *Ae. aegypti* mosquitoes fed within four hours using the glass feeder, compared to 85.9% with the Hemotek system. Additionally, mosquitoes fed using glass feeders laid 16% more eggs than those fed with Hemotek. These findings demonstrated that the glass membrane feeder is more efficient than Hemotek in both feeding success and egg productivity. Furthermore, the glass feeder was more cost-effective and can simultaneously accommodate multiple cages, making it a practical option for mass mosquito rearing facilities. Overall, these results provided valuable insights to guide insectaries in selecting efficient blood-feeding systems to enhance mosquito colony sustainability and expansion for SIT applications.



Comparison between glass feeding system and Hemotek apparatus for *Aedes aegypti* colonies

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INTRODUCTION

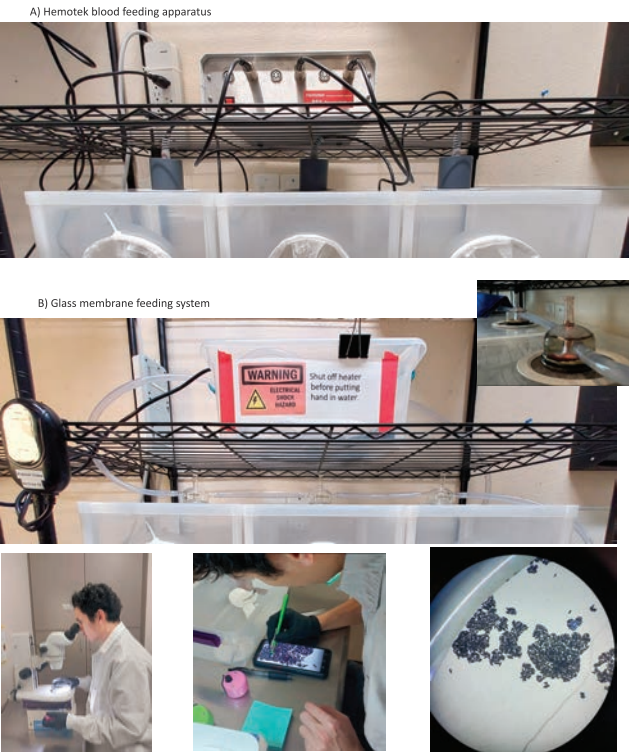
The Sterile Insect Technique (SIT) relies on maintaining healthy mosquito colonies in insectaries, which in turn depends on efficient blood-feeding methods to ensure consistent egg production—particularly for mass rearing and sustained sterile male releases. Optimizing feeding procedures not only supports colony health but also enhances operational efficiency and resource utilization. This study compared the performance of two mosquito blood-feeding apparatuses—the Hemotek feeding system and the glass membrane feeder—to evaluate their relative efficiency in supporting *Aedes aegypti* colony maintenance.

MATERIALS AND METHODS

This study was conducted at the West Valley Mosquito and Vector Control District.

Triplicates of cages for the two cohort – Hemotek apparatus and glass feeder – were set up. Each cage contained a total of 200 freshly emerged female and 200 male *Ae. aegypti* mosquitoes. After a week of mating, females were blood fed using bovine blood via either of the two blood feeding systems (Figure 1). Blood feeding was carried out for four hours for two days. Number of engorged females were recorded after each blood feed. Mortality was recorded daily (Figure 2). Eggs were collected the following week and counted.

Figure 1. Blood feeding set up: A) Hemotek blood feeding apparatus; B) Glass membrane feeding system



RESULTS

- Results showed that 88.6% of female *Ae. aegypti* mosquitoes fed on the first day using glass feeders, compared to 85.9% with the Hemotek apparatus (Table 1). The difference however was not statistically significant ($P = 0.211$)
- Mosquitoes fed using glass feeders laid 16% more eggs than those fed with Hemotek (Table 2). The difference however was not statistically significant ($P = 0.105$)
- Mortality rate was slightly higher in the cages fed using Hemotek apparatus compared to glass feeders ($P > 0.05$).

Figure 2. Mortality rate (%) in experimental cages

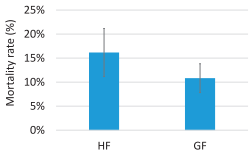


Table 1. Number of bloodfed mosquitoes using Hemotek apparatus and glass feeding system.

Blood feeding system	Cage	Number of females in the cage	Number of bloodfed mosquitoes	Mean	P value
Hemotek	HF 1	200	172	171.8	0.211
	HF 2	200	181		
	HF 3	200	179		
Glass feeder	GF 1	200	171	177.1	
	GF 2	200	165		
	GF 3	200	180		

Table 2. Mean number of eggs collected per *Ae. aegypti* mosquito after blood feeding with the Hemotek feeding apparatus and glass membrane feeder system.

Blood feeding system	Cage	Number of eggs collected per mosquito	Mean	P value
Hemotek	HF 1	55	62.6	0.105
	HF 2	62		
	HF 3	72		
Glass feeder	GF 1	72	73.2	
	GF 2	77		
	GF 3	70		

CONCLUSIONS

- The findings demonstrate that the glass membrane feeder is more efficient than Hemotek apparatus in both feeding success and egg productivity.
- Furthermore, the glass feeder is more cost-effective and can simultaneously accommodate multiple cages, making it a practical option for mass mosquito rearing facilities.
- Overall, these results provide valuable insights to guide insectaries in selecting efficient blood-feeding systems to enhance mosquito colony sustainability and expansion for SIT applications.

A tale of two trays: fry tray design affects collection yield

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Introduction

Mosquitofish are live-bearing and cannibalistic, therefore high-density aquaculture often leads to significant loss of fry. To improve yields, a simple floating fry tray was designed to shield birthing females and provide a refuge that protects fry and simplifies their collection. Fry trays (T) and pods (P) were evaluated to determine if design and spatial position affects fry yield.

Methods

Experimental Design and Tank Configuration

The study was conducted using three 9,463 L (2,500 gal) circular tanks, designated as tanks 1, 2, and 3. Each tank was partitioned into four quadrants (A, B, C, and D) labeled in a clockwise orientation. To standardize the spatial orientation relative to the water inlet and flow, quadrant A was positioned immediately clockwise to the water inlet. Fish age and stocking densities were maintained as independent variables across the three replicates.

Collection Vessel Specifications

Two distinct vessel designs were evaluated, trays (T) (Fig. 1) and pods (P) (Fig. 2). Four collection vessels (2T and 2P) were deployed per tank, with one vessel situated in each quadrant.



Figure 1.—Fry tray (T).

- Tray (T): Constructed 2' × 4' rectangular PVC frame (2" diameter) providing buoyancy, utilizing black 1/8" plastic mesh and 1/2" plastic egg crate, with each tray featuring many 6"–8" suspended garland lengths, providing a total surface area of 6.11 ft². (Fig. 1)
- Pod (P): Utilized a design similar to the tray (T) but encased between two 7-gallon plastic mortar pans with cutout sides and tops. Buoyancy was maintained by two 24" × 2" PVC floats, resulting in a total surface area of 2.67 ft². (Fig. 2)

Sampling Protocol and Fry Management

Data collection occurred from June through August 2025 (N = 26 sampling events). Fry were collected daily Monday through Friday from each vessel, counted, and transferred to dedicated grow-out tanks. Following the daily collection and counting process, vessels were rotated one quadrant clockwise within each tank to mitigate potential bias associated with tank position or inlet proximity.

Results

A total of 10,539 *Gambusia affinis* fry were collected, with pods (P) collecting more fry than trays (T) (Fig. 3). Relative to the position of the water inlet, the number of fry collected from trays (T) across positions A through D was relatively stable, although positions C and D produced slightly higher total counts. The number of fry collected



Figure 2.—Fry pod (P).

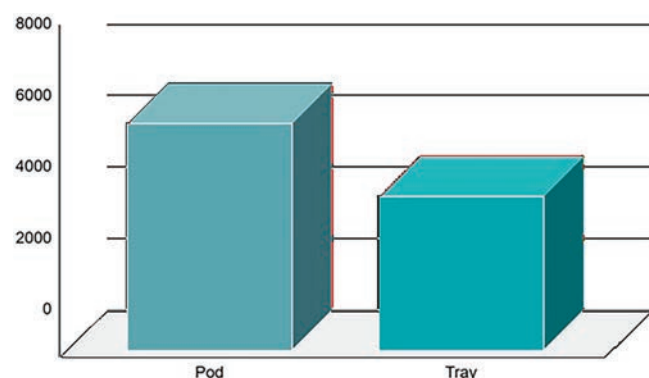


Figure 3.—Total fry collection for each collection style.

from pods (P), relative to the water inlet, was considerably higher from every position in the tank when compared to trays (T). A similar upward trend in counts was seen from pod (P) positions C and D, with position D producing the highest yield (Fig. 4). When normalized for collection surface area, a T-Test revealed that pods (P) collected > 3 times more fry than trays (T) (77.3% vs. 22.7%; $P < 0.0001$). Although not statistically significant (ANOVA, $P = 0.066$), positions C and D collected more fry than positions A and B when normalized mean counts were combined for both style P and style T.

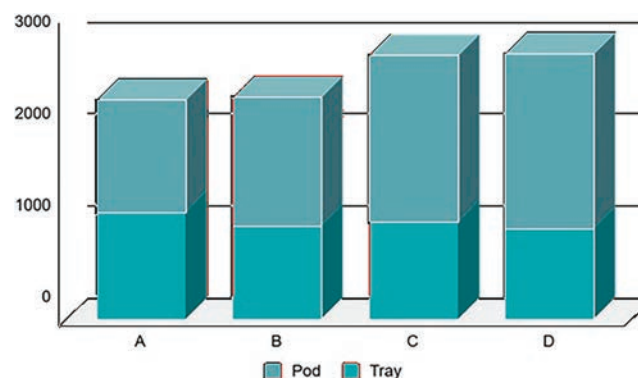


Figure 4.—Total fry collection by position (A-D) and style (P, T).

Conclusions

We conclude that pods (P) were a more effective design for maximizing collection yield, and position D represented a locally optimal placement in the tanks relative to water inlet and flow.

Acknowledgement

The author wishes to thank the Laboratory staff at the Contra Costa Mosquito and Vector District for their assistance with Aquaculture and greenhouse maintenance.

Predators as Sentinels: Monitoring anticoagulant rodenticide exposure in live carnivores

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Abstract

Urban and peri-urban predators offer a unique window into environmental contamination from anticoagulant rodenticides (ARs). We present findings from a multi-year study examining exposure in live-captured coyotes (*Canis latrans*) across Southern California. Using blood, hair, and scat samples, we detected widespread residues of both first- and second-generation ARs, highlighting the persistence of these compounds in local food webs despite evolving regulatory restrictions (e.g., AB 1788, AB 1322, AB 2552). By integrating residue analysis, stable isotope diet reconstruction, and GPS telemetry, we identified spatial and behavioral patterns linked to higher exposure risk, such as proximity to urban edges and specific land-use types. Our results underscored the importance of live-animal monitoring to complement traditional carcass-based surveys, allowing earlier detection of sublethal exposure and informing adaptive management strategies that protect both wildlife and human communities.

From Boxes to Behavior: Quantifying rodent interactions with bait stations to improve urban rodent management

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Abstract

Understanding how rodents interact with bait stations is critical for developing effective and environmentally responsible management strategies. Building on earlier work documenting non-target wildlife visitation at suburban bait stations in Orange County, our most recent study extends this foundation using a high-resolution, motion-triggered camera system and enhanced behavioral metrics. We evaluated roof rat (*Rattus rattus*) discovery, entry, and activity patterns across bait stations of varying design, placement, and environmental context. Results indicated that rats did not interact with bait stations as we previously thought leading to further study where we aimed to improve the use of bait stations. Together, these results inform best-practice guidelines for urban pest management professionals, emphasizing data-driven deployment strategies that balance control success with wildlife conservation.

Hantavirus surveillance and prevention efforts in Yosemite National Park, 2019–2025

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Abstract

Sin Nombre virus (SNV), the causative agent of hantavirus pulmonary syndrome (HPS), is carried primarily by deer mice (*Peromyscus maniculatus*), which are abundant in many regions of California. SNV can be transmitted to humans through the aerosolized droppings, urine, and saliva of infected deer mice. Following an outbreak of 10 hantavirus cases associated with visits to Yosemite National Park in 2012, the California Department of Public Health, Vector-Borne Disease Section (VBDS) has coordinated with the National Park Service to conduct regular deer mouse surveillance and facility evaluations in Yosemite to help assess hantavirus risks and implement prevention measures. In our presentation, we summarized surveillance conducted from 2019 through 2025 and compared findings to the post-outbreak period (2013–2018) previously summarized by Danforth et al. (2022). In Yosemite Valley locations, outdoor deer mouse trap success increased from 9% to 16% and SNV seroprevalence increased 5% to 14% between these time periods. In comparison, both trap success (34%, 2013–18; 24%, 2019–2025) and SNV seroprevalence (15%, 2013–18; 20%, 2019–2025) were higher at Tuolumne Meadows locations than at Yosemite Valley locations for both time periods. The highest outdoor trap success rates and seroprevalence levels in Yosemite were found between 2023 and 2025, reflecting trends seen in other areas of the state. These recent increases prompted VBDS staff to perform additional facility evaluations to improve existing rodent exclusion and control measures inside park buildings. The recent increases in deer mouse abundance and SNV prevalence have highlighted the importance of ongoing rodent surveillance, facility evaluations, and public education efforts to help reduce hantavirus risks for Yosemite staff and visitors.

Best Management Practices for feral cats in communities with flea-borne typhus

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Abstract

The mission of the Orange County Mosquito and Vector Control District (District) is to protect public health by educating the community and controlling vectors and vector-borne diseases in an environmentally responsible manner. The District responds to flea-borne typhus (FBT) cases, flea-related service requests in public areas, and FBT - positive flea test results by conducting site inspections and providing recommendations to reduce flea populations and eliminate conditions conducive to infestation. Prevention of flea-borne typhus relies primarily on minimizing human exposure to fleas. In recent years, feral, free-roaming, and unowned domestic cats have been identified as significant reservoirs supporting flea populations infected with the pathogens responsible for flea-borne typhus, cat flea rickettsiosis, and cat scratch disease. The establishment and maintenance of cat colonies, especially where cats are intentionally released and provisioned at feeding stations, can amplify flea abundance and increase the risk of human-vector contact and infection with flea-borne pathogens. To address these public health concerns, the District has developed Best Management Practices (BMPs) for feral cat management in communities affected by flea-borne typhus. These BMPs provide clear guidance to municipalities, animal care agencies and organizations, residents, and other stakeholders on strategies to reduce flea infestations and limit disease risk. The District encourages jurisdictions and partner organizations in affected areas to adopt policies and public messaging aligned with these BMPs to effectively safeguard both human and animal health.

Introduction: Flea-borne Typhus, Fleas, Urban Wildlife, and Feral Cats: An Increasing Public Health Threat

From 2016 to 2025, more than 1,800 cases of flea-borne typhus [FBT; includes the etiologic agents *Rickettsia typhi* (murine typhus) and possibly *R. felis* (cat flea rickettsiosis)] were reported to the California Department of Public Health (CDPH), making it the second most common vector-borne disease in the state after West Nile virus (CDPH 2025). In 2025, FBT became the most reported vector-borne disease in California, with more than 300 cases, the majority occurring in Southern California (CDPH 2026). In Orange County, the incidence of FBT continues to increase, with 38 human cases reported in 2025, the highest annual total recorded for the county (CDPH 2026).

Flea-borne typhus presents significant challenges for public health management because transmission is tightly linked to human behavior and a complex vector-host dynamic involving domestic pets, free-roaming and feral cats, wildlife, and fleas (Cummings et al. 2022). The ecology of fleas and their hosts, combined with the lack of effective orally administered flea control (feed through) for wildlife and feral cats, limits the availability of flea control strategies (Krueger et al. 2022).

For communities participating in trap-neuter-return (TNR), return-to-field (RTF), return-to-home (RTH), or similar community cat sterilization and release programs, where unowned cats are released outdoors after capture or

processing, routine flea control on the released cats is rarely maintained. There are no approved oral flea control products in the United States that can be reliably delivered via bait or food to free-roaming cats or wildlife. Cat colony feeding practices further exacerbate FBT risk by providing supplemental food that attracts and aggregates multiple host species, creating focal points for flea amplification and interspecies pathogen exchange (Akucewich et al. 2002; Hernandez et al. 2018). Additionally, it is estimated that approximately 20% of residents feed feral cats, contributing to sustained cat populations and increasing the likelihood of flea infestations and pathogen transmission in affected neighborhoods (UC IPM 2023).

Best Management Practices for Feral Cats in Communities with Flea-borne Typhus

Following the re-emergence of FBT in Orange County in 2006, after approximately two decades without reported cases, the District partnered with academic researchers and public health agencies to investigate the ecological drivers of disease transmission and enhance the District's laboratory diagnostic capacity. These efforts focused on identifying environmental and epidemiological factors associated with increased human risk (Abramowicz et al. 2010; Eremeeva et al. 2012; Krueger et al. 2013, 2016; Maina et al. 2016; Cummings et al. 2016, 2022; Mullins et al. 2018; Penicks et al. 2019, 2020; Li et al. 2021; Driscoll et al. 2020).

The results of these collaborations enabled the District to identify key FBT risk factors, including establishment of feral cat colonies and TNR programs by local animal welfare organizations in densely populated areas with abundant synanthropic wildlife (Cummings et al. 2014, 2016, 2022); improper handling of dead wildlife; lack of flea control on domestic pets; and environmental conditions that support animal harborage. With this information, the District has delineated high-risk FBT areas and implement targeted education, prevention, surveillance, and response strategies for affected areas (OCMVCD VRP 2010; Cummings et al. 2022; Quinn et al. 2019; Krueger et al. 2022).

Agency-sponsored feral cat releases in FBT-endemic areas represent a complex social and behavioral challenge that cannot be mitigated through conventional vector control measures alone. To minimize the public health risks associated with releasing TNR-processed feral cats in Orange County, the District developed a set of Best Management Practices (BMPs) for communities affected by feral cats, fleas, and FBT. These BMPs were designed to provide systematic, outcome-oriented steps for resolving feral cat and FBT issues. Ideally, the District's BMPs emphasize behavior-based interventions aimed at reducing human health risks and environmental impacts from feral cat colonies. These strategies are structured around a harm reduction framework - a public health strategy that focuses on minimizing adverse outcomes by modifying human behavior rather than attempting to eliminate the underlying activity entirely.

The BMPs for Feral Cats document is a foundation for the District's Vector Reduction Program and is applied when coordinating abatement activities at properties, or with individuals, responsible for conditions deemed as a Significant Vector Source (OCMVCD 2010). The BMPs are distributed to property owners and managers, animal care agencies, animal rescue and welfare organizations, and other stakeholders to provide standardized guidance for reducing conditions that promote flea infestations and FBT transmission associated with outdoor cats and cat feeding practices (OCMVCD 2010). The BMP document is publicly available on the District's website and serves as a resource for communities and governmental agencies in areas affected by feral cat feeding and FBT (OCMVCD 2023).

The District's Best Management Practices (BMPs) for Cats in Flea-borne Typhus (FBT) Affected Communities

1. Programmatic Requirements for Cat Releasing Organizations

Local animal care agencies and rescue organizations that release cats into Orange County neighborhoods should establish and implement written guidelines that include:

- a) Public education programs addressing the risk of flea-borne typhus and the importance of year-round flea control for pets;

- b) Training for staff and volunteers on site restrictions for cat releases, consistent with BMP Items 2–6;
- c) A formal mechanism for residents to report concerns related to fleas and feral cats;
- d) Defined procedures for timely investigation and resolution of complaints; and
- e) Routine monitoring and periodic review of release sites to ensure compliance with BMPs.

2. Jurisdictional Approval

Cats should not be released within any city jurisdiction without prior approval from the local governing authority, in accordance with applicable municipal policies.

3. Sensitive Site Buffer Zones

To protect public health, environmental resources and water quality, cats should not be released within a 0.3-mile radius of sensitive land uses or land with policies prohibiting free-roaming animals. These areas include mobile home communities, hospitals, convalescent facilities, schools, childcare centers, workplaces, and designated public lands such as beaches, parks, and flood control channels.

4. Disease Risk Buffer Zones

Cats should not be released within a 0.3-mile radius of documented human flea-borne typhus exposure sites. In the event of a disease cluster (defined as two or more human cases occurring within a 0.5 square-mile area), cat releases should be prohibited throughout the entire cluster area.

5. Colony Density Management

Cats should not be released into areas where existing cat colonies or feeding activities are already present, to avoid increasing colony size and associated vector risks.

6. Property Owner Consent

Cats should not be released onto private property without explicit permission from the property owner.

7. Licensing and Tracking

Animal care agencies and local governments should encourage cat licensing requirements to facilitate tracking and monitoring of released cats, colony locations, and ongoing management activities.

8. Ordinance Enforcement

Animal care agencies and local governments should enforce applicable ordinances that promote responsible pet ownership and protect animal welfare.

Because the release of cats can result in measurable environmental impacts, the District's BMPs include guidance recommending compliance with the California Environmental Quality Act (CEQA). CEQA requires state and local agencies to identify significant environmental impacts associated with discretionary projects and to avoid or mitigate those impacts where feasible (LCI 2025).

Consistent with this framework, the District BMPs recommend that local governments evaluate whether cat release activities constitute a project under CEQA and, if so, whether the associated environmental effects warrant formal review (LAC CCP 2020). The CEQA process is governed by the statute (Public Resources Code §21000 et seq.), the CEQA Guidelines (California Code of Regulations, Title 14, §15000 et seq.), relevant case law interpreting CEQA, and locally adopted CEQA procedures (LCI 2025).

Legal Authority of Mosquito and Vector Control Districts

The California Health and Safety Code, Division 3, Chapter 1, Sections 2000–2093 (Mosquito Abatement and Vector Control District Law) establishes the legal authority for vector control districts to conduct surveillance, prevention, abatement, and control of vectors that transmit disease or create public nuisance conditions. This authority is further supported by guidance from CDPH and the California Department of Fish and Wildlife (CDFW), exempting mosquito and vector control districts from obtaining a scientific collection permit when collecting vectors as part of official surveillance activities (CDPH and CDFW 2015). Additional clarification is provided by CDPH in the *Guidance for Flea-borne Typhus Surveillance and Reporting*, which affirms district authority to investigate and respond to properties that create conditions conducive to flea-borne typhus transmission (CDPH 2019).

Human cases of vector-borne disease are reported to the Orange County Health Care Agency (OCHCA) and shared with the District in accordance with procedures established in a Memorandum of Understanding (MOU) (OCHCA and OCMVCD 2012). Under this agreement, all District staff receive annual training in Health Insurance Portability and Accountability Act (HIPAA) compliance. The MOU allows the District, at its discretion, to share relevant information with local animal care agencies when necessary to support investigations and response activities at suspected exposure sites.

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Impacts of the Eaton fire on mosquito abundance, species diversity, and distribution in the San Gabriel Valley

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Abstract

In January 2025, the Eaton wildfire destroyed thousands of homes in the San Gabriel Valley. The San Gabriel Valley Mosquito and Vector Control District (SGVMVCD) responded with particular focus on the thousands of damaged, unmaintained swimming pools in the area. Existing surveillance sites and control practices allowed for effective response through a rapidly evolving emergency.

Weekly surveillance of historical gravid trap sites was supplemented with additional gravid traps, BG Sentinels, and emergence traps in and around the affected area. The historic sites provided a baseline of expected *Culex quinquefasciatus* abundance and arboviral infection rates. Although *Cx. quinquefasciatus* abundance remained within historical averages, other WNV vector species increased in abundance compared to historical data and to the rest of the District. Emergence traps and CO2-baited BG Sentinels reflected shifts in abundance and distribution of several *Culex*, *Anopheles*, and *Aedes* species typically associated with wetland habitats.

The results of the SGVMVCD response to the Eaton Fire demonstrated how historical data can be adapted to respond to an atypical emergency that is not flood-related. The ecological impacts of the Eaton Fire will last for years, as elucidated by the continued analysis of routine surveillance data.

Eaton Fire Field Operations: Innovation through teamwork in post-fire mosquito control

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Abstract

In early 2025, the Eaton Fire burned through residential areas within the San Gabriel Valley MVCD (District) service boundary, destroying infrastructure and leaving thousands of properties uninhabitable. Among the secondary effects was the creation of widespread mosquito larval habitat in abandoned and unmaintained swimming pools. District staff faced the challenge of identifying, prioritizing, and treating pools that posed the highest vector risk—all while maintaining regular operations across the service area.

To meet this challenge, the Operations Department developed an adaptive post-fire control plan grounded in teamwork, collaboration, and innovation. Using Nearmap data, staff filtered and classified impacted properties, ultimately identifying over 1,400 pools requiring treatment. The team designed and tested cost-effective floating treatment devices using mesh bags and pool noodle floats, enabling rapid and efficient larvicide deployment. Collaboration with the Greater Los Angeles County Vector Control District supported the first treatment round, with subsequent operations completed independently by District field staff using Natular NXRT, Altosid XR, and *Gambusia* fish. The Eaton Fire response demonstrated how teamwork and innovation can transform an overwhelming task into an effective and sustainable mosquito control program.

CDPH support during the Eaton Wildfire mosquito response

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Abstract

The California Department of Public Health (CDPH) holds the primary responsibility for supporting vector control mutual aid resource requests at both regional and state levels, as described in the California State Emergency Plan. This is within the “Public Health and Medical” support function also known as Emergency Support Function #8. During the Eaton Fire, the San Gabriel Valley Mosquito and Vector Control District (SGVMVCD) had both the expertise and authority to address the wildfire-induced, vector-borne disease risk, but required additional resources for their response efforts.

The CDPH Center for Environmental Health and the Center for Infectious Disease’s Vector-Borne Disease Section supported SGVMVCD by opening lines of communication, bringing key emergency management partners together, advocating at the state level, and supporting SGVMVCD in procuring the necessary resources to effectively respond to the wildfire.

San Gabriel Valley MVCD Easton Fire FEMA Project Submission

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The Federal Emergency Management Administration (FEMA) Public Assistance (PA) Program aids Public Entities and certain Private Non-Profit organizations (PNPs) who have requested public assistance for costs incurred in response to a Federally declared disaster.

In the wake of the Eaton Fire there were approximately 2,800 properties destroyed or evacuated which contained swimming pools which were left unattended. These pools quickly became productive sources for mosquitoes. In response to this infestation, The San Gabriel Valley Mosquito and Vector Control District (SGVMVCD) deployed staff to chemically treat these pools and destroy the mosquito habitats.

Following the Presidential Disaster Declaration, SGVMVCD submitted a request for reimbursement for funds allocated to eradicate this threat. The Policy administered, which outlines the required processes and documentation required to accept FEMA reimbursement is summarized in FEMA's Public Assistance Program and Policy Guide (PAPPG). Appendix F of this guide outlines the required criteria for reimbursement of costs for Mosquito Abatement activities.

Project Submission

The SGVMVCD submission was distinctive, as the process for chemically treating mosquito larvae in swimming pools varied from the required criteria outlined in the PAPPG for reimbursement. Appendix F requires the presence of adult mosquitoes and measured disease, as baseline criteria to be recognized as a threat to public health and thus qualify as a reimbursable activity.

It was the mission of SGVMVCD to preemptively thwart the threat of disease before the mosquitoes matured and entered the community. There were two projects developed for submission to FEMA, abatement efforts in swimming pools inside the burn scar and outside the burn scar. The

approach taken for submission of these projects leveraged the PAPPG recognition that mosquito abatement is a reimbursable effort, due to the health threat posed to the community and the acceptance of larval treatment measures in response to that threat.

The challenge for this submission, unlike most project submissions, was not focused on scope, cost and supporting documentation. This submission effort would require focus to redefine the health threat without the presence of adult mosquitoes and measured arbovirus infection in those mosquitoes.

To this end, a scientific summary report was needed to clarify this particular health risk to the community.

The Report

With the cooperation of the Los Angeles County Health Officials, data was provided to support the presence of disease in the community. SGVMVCD provided data to identify the quantity and species of mosquito larvae present in the pools. This report, which summarized these data and additionally considered the large, at-risk population, was provided to FEMA in support of the SGVMVCD project submissions. This report, consistent with FEMA policy, was reviewed by the Center for Disease Control (CDC) as part of the normal FEMA process for health-related projects.

Following their review of this SGVMVCD report, the CDC supported the SGVMVCD risk assessment and approach to abatement. With CDC concurrence of the threat to the community, the projects were submitted to FEMA outlining the scope and cost for the SGVMVCD approach to eradicate this threat.

FEMA accepted the submission and subsequently obligated (funded) both projects. The efforts to treat swimming pools at homes completely destroyed inside the burn scar; and additionally, pools located at properties damaged or temporarily abandoned outside the burn scar, were approved for reimbursement.

After the Storm: Managing FEMA and CalOES recovery for Mosquito and Vector Control Operations

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Abstract

On April 3, 2023, President Biden declared a major disaster, making federal disaster aid available to multiple California counties affected by severe winter storms, straight-line winds, flooding, landslides, and mudslides. Special Districts—including Mosquito and Vector Control Districts—became eligible for Category B (emergency work) and Category Z (management costs) public assistance funds.

For Fresno County, the incident period began February 21, 2023, with the deadline for requests for public assistance set for May 14, 2023. But how do you apply for disaster assistance when the true mosquito and vector control impacts of that disaster have yet to emerge?

This presentation shared the Consolidated Mosquito Abatement District's experience navigating the FEMA and CalOES public assistance process—from initial application through reimbursement and beyond. Mosquito control expenses directly related to excess flooding and irrigation were eligible for reimbursement; however, the disbursement of FEMA funds to the District was not the final step in the FEMA Public Assistance reimbursement process. This session highlighted the unexpected follow-up requirements, lessons learned, and key takeaways to help districts prepare for the ongoing challenges of post-disaster funding compliance.

Records Retention: Keeping What Matters and Letting Go (*Knowing what to keep and when to securely discard keeps the District organized, compliant, and ready for audits.*)

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Abstract

Public agencies generate an enormous amount of information and knowing what to keep and what to securely discard is essential for legal compliance, operational efficiency, and public trust. The current session provided a practical overview of records retention for vector control districts, with a focus on helping staff at all levels understand how to identify, manage, and protect official records. Participants learned what qualifies as a record, how long different types of documents must be preserved, and why these requirements exist beyond simply hearing “because admin says so.”

The presentation described the common categories of records encountered in field operations, administrative functions, and interdepartmental communication. It also highlighted strategies for proper storage, secure disposal, and preparing for audits. By simplifying complex retention rules into approachable tasks, this session equipped emerging leaders, field staff, and support personnel to make informed decisions when handling documents and data. Ultimately, attendees obtained clear guidance on how to support agencywide compliance and contribute to a more organized, accountable, and audit-ready district.

Safety Committees: Turning Conversations into Safer Workplaces (*How employee-led safety groups make a real difference in daily operations*)

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Abstract

Safety committees are an important part of a public agency's responsibility to prevent injuries and promote a workplace where staff feel informed, supported, and protected. This session explained how safety committees function within a vector control district, including how they are formed, what topics they review, and how recommendations move from discussion to implementation. The presentation highlighted the role of employee participation and why contributions from both field and administrative staff are essential to maintaining safe operations.

Attendees learned how safety committees can influence policy development, training improvements, and hazard prevention efforts. The session also covered communication strategies that help staff understand how their feedback shapes real changes in the workplace. By the end of the presentation, participants understand how consistent, employee-driven conversations can build stronger safety practices and help ensure everyone goes home safe each day.

Mosquito Wars: Finding funds to fight the World's deadliest animal in coastal California

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Abstract

Mosquito and vector control in California is entering a high-stakes transformation. Invasive *Aedes aegypti* are here; longer insect seasons are driven by climate change, and emerging technologies - from releasing sterile mosquitoes to drone surveillance - are reshaping operational realities for many districts. These high-tech tools are promising, but come with high costs. At the same time, many of California's Mosquito and Vector Control districts rely on revenue from benefit assessments established 20–25 years ago that no longer match the realities of modern mosquito control. Funding levels that once fit district needs are now strained. As new technologies become viable options, districts must find new ways to raise revenue to respond to emerging vectors and proactively protect public health.

Bats are Friends, Not Foes

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Threat of Rabies

California is home to around 25 species of bats, which are often feared for their potential to transmit rabies. Confirmed human rabies cases have been extremely rare in the United States since the 1940s, due to pet vaccination requirements and post-exposure prophylaxis protocols (Held et al. 1967). As a result, rabies in California is primarily associated with wild mammals, especially bats and skunks. Although bats represent the highest *number* of rabies-positive wild mammals in California each year, it is important to highlight that bats also represent about 86% of all wild mammals submitted for testing. Furthermore, the percentage of bats that test positive has remained stable (10-13%) between 2012 and 2023 (California Department of Public Health, 2025). Because of robust surveillance and treatment options in the United States, only 15 domestically acquired cases of human rabies were documented between 2015 and 2024, an average of just 1.5 cases per year (U.S. Centers for Disease Control and Prevention, 2025). Additionally, when collected for mortality studies unrelated to rabies surveillance, less than 1% of deceased bats test positive for rabies, as opposed to bats that are euthanized and submitted to public health agencies after potential human or animal exposure (Klug et al. 2011).

Benefits of Bats

Considering these statistics, the positive ecological and economic impacts of bats outweigh their likelihood of spreading disease. Some studies have estimated that insectivorous bats save the global agriculture industry billions of dollars each year by consuming crop-destroying pests (Maine and Boyles 2015, Tuneu-Corral et al. 2023), whereas fruit and nectar-feeding bats play critical roles in regenerating clear-cut forests and pollinating over 500 plant species, including cash crops and medicinal plants (Bat Conservation International, 2026). Numerous studies also have shown that insectivorous bats are highly opportunistic and their diets include vectors, such as mosquitoes (Hughes et al. 2022, Puig-Montserrat et al. 2020, Wray et al. 2018). In addition, their unique physiology has contributed to advancements in medicine and biotechnology (North American Bat Monitoring Program, 2018). Bat guano sustains cave ecosystems (Sakoui et al. 2020) and is used to study climate science (Cleary et al. 2018). Some local economies benefit from bat-driven ecotourism. However,

bat populations around the world face serious threats, including habitat loss, climate change, pesticide use, human exploitation, wind turbines, and a highly contagious fungal disease known as white nose syndrome (Adams et al. 2024, Oliveira et al. 2021). To protect these fascinating creatures that are vital to science, agriculture, and native ecosystems, it is imperative for vector control personnel to educate the public on the importance of bats while also providing sensible advice and services to prevent the transmission of rabies.

Response to Bat Encounters

Understanding bat ecology and rabies transmission can help dispel misguided fears and guide professionals in evaluating the risk of rabies exposure. Individuals who encounter bats should always seek prompt medical attention and contact their local public health agency when the following criteria are met: there is a known bite or scratch from the bat; the bat was handled with bare hands; or the bat was trapped in a room with a person who cannot say with certainty that they did not have contact with the bat (e.g., they were asleep, have impaired cognition, or are a young child). Animals should be taken for veterinary attention if they may have come in contact with a bat and are not vaccinated for rabies. Finally, if the bat is still present, it should be reported to the local animal control agency for capture and submission for rabies testing.

If the bat encounter did *not* result in any of the situations described above, there is no immediate need to euthanize the bat for rabies testing. In these cases, the best option is to contact a permitted wildlife rehabilitator. They will determine if the bat should be left alone, carefully relocated, or safely captured and brought to them for medical attention. If the bat must be handled, they will provide instructions for using personal protective equipment to prevent direct contact with the bat. Previous experience with bat surveillance and wildlife rehabilitation has taught the author (AE) that there are many situations in which trapped or injured bats can be rescued and released back to the wild. Like vector control agencies, permitted wildlife rehabilitators have a duty to protect public health, and they will also assess the risk of rabies exposure before accepting a bat into rehab. If a bat in their care is suspected of having rabies, they will take all the proper steps to report potential exposure and submit the bat for testing. California Department of Fish and Wildlife maintains a list of

permitted wildlife rehabilitators on their website, and Bat World Sanctuary provides an interactive map of bat rehabilitators around the world. Most wildlife agencies in other states also provide their own lists of permitted rehabilitators. For general advice about the safe removal and exclusion of bats from buildings, Bat Conservation International is a great resource.

Coexisting with Bats and Other Rabies Vectors

Aside from responding to bat encounters, vector control professionals can help protect bats and public health by educating the public to never handle or approach wildlife, keep pets and domestic animals vaccinated, and make sure homes and buildings are well-sealed to exclude wildlife. Allowing pets to roam free or unsupervised should be discouraged, especially cats, as they are most likely to catch bats and bring them indoors (Hormick, Bat Conservation International, 2025). It is also good practice to remind people not to reach hands into tight hidden spaces where animals could be hiding. Teaching sensible wildlife etiquette and knowing when it is truly necessary to test a bat for rabies can potentially save hundreds of bats each year and return them to the ecosystems and industries that rely on their services.

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More than mosquitoes and fire ants: Arachnids of the Coachella Valley

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The Coachella Valley Mosquito and Vector Control District (CVMVCD) primarily focuses its control efforts against mosquitoes and red imported fire ants. However, there are a few other invertebrates of medical significance found in the Coachella Valley. Occasionally residents will call the District or talk to employees about some of the arthropods they encounter. One such group is the arachnids. The Coachella Valley has an abundance of spider and scorpion species. The western black widow (*Latrodectus hesperus*) and desert recluse (*Loxosceles deserta*) are two species of spiders that have venomous bites. There are several spiders that are misidentified as western black widows and recluse spiders. The careful inspection of identifying features can help distinguish the venomous spiders from their non dangerous counterparts. Scorpions are another group of arachnids found throughout the Coachella Valley. Although the majority of scorpions in the valley are harmless, the invasive Bark Scorpion (*Centruroides sp.*) has a medically significant sting. This scorpion has been found in isolated neighborhoods in Indio and Palm Desert. Understanding the Bark Scorpions habits and ecology may help prevent stings as they become more common in the Coachella Valley.

The western black widow is common in the Coachella Valley. It can be easily identified by its glossy black body and infamous red hourglass on the underside of its abdomen. Some spiders can be misidentified as black widows through superficial appearances such as the brown widow (*Latrodectus geometricus*) and the false black widow (*Steatoda grossa*). Western Black Widows can be found in several different habitats in urban environments. Their webs have no distinct pattern and tend to be closer to the ground. The bite of the Western black widow is neurotoxic and has an array of symptoms such as rigid stomach, urine retention, and localized pain. Although bites can be serious, they are usually rare (Vetter 2017).

Recluse spiders are found throughout the southern parts of the United States. The brown recluse (*Loxosceles reclusa*) is not native to the Coachella Valley; however the desert recluse is found in the rural parts of the valley. Many spider families can be confused with recluse spiders due to their brown coloration. Desert recluses and other recluse spiders can generally be identified by their eye arrangement, lack of long hairs, lack of long spines on legs, brown coloration,

and violin shape marking in the carapace. These spiders are renowned for their “necrotic bite.” Although necrosis is possible, it is a rare occurrence from recluse spider bites (Vetter 2005). Researchers have created a mnemonic device, “NOT RECLUSE,” to help individuals’ diagnosis their ailments as possible recluse bites (Stoecker et al. 2017).

Bark scorpions are found in the deserts of Arizona. In 2014, there were reports of bark scorpions appearing in the Coachella Valley (White et al. 2015). Unlike native species of scorpions, the bark scorpion has a long slender build and has a tooth like protrusion on the telson right above the stinger. They have been found in isolated neighborhoods in crevices of cinderblock. The sting of the bark scorpion is the only medically significant scorpion sting in the United States. Although the sting can lead to hospitalization, stings are rare (Natwick 2011).

Many conventional control methods do not work for arachnids. The best methods for control is habitat reduction. Homeowners should keep yards and garages tidy and free of hiding spots for these arachnids. Caulking and sealing cracks is also encouraged. (Natwick 2011) Although these arachnids can potentially give medically significant bites/stings, death from arachnid interactions is extremely rare (U.S. Scorpion Exposure Reports (1999–2023) 2025).

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What's crawling in California: Ticks and tick-borne diseases

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Abstract

The Vector-Borne Disease Section (VBDS) of the California Department of Public Health maintains a comprehensive statewide environmental tick surveillance and testing program. This presentation addressed the vectors, pathogens, and countywide prevalence of these pathogens in tick vectors throughout the state, summarizing data from 2021 through 2025. Also addressed were recent nomenclature changes related to ticks and tick-borne diseases, and emerging tick-borne pathogens in California.

OCMVCD's Tick surveillance program-from flagging to noosing lizards

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Abstract

Tick-borne disease (TBD) surveillance is an integral component of the multi-vector surveillance program at the Orange County Mosquito and Vector Control District (OCMVCD). Several tick species capable of transmitting *Borrelia burgdorferi* and *Rickettsia rickettsii* are present in Orange County, California. Although rare in the region, TBDs are detected intermittently, with the most recent occurrence in 2022. A revised surveillance strategy was developed in 2023 and implemented in 2024 to align with updated VectorSurv data parameters and improved site selection. Perennial sites are maintained based on historical tick activity, pathogen detections, and high public exposure risk. In addition to perennial sites, OCMVCD includes new and existing sites in a rotating three-year cycle to ensure broad regional coverage. Active surveillance is conducted primarily through flagging, while immature ticks are also collected via lizard noosing *Sceloporus occidentalis* and *Elgaria multicarinata* lizards. These enhanced surveillance efforts contribute to regional species documentation, pathogen detection, and early recognition of potential invasive tick species.

Introduction

The Orange County Mosquito and Vector Control District (OCMVCD) has conducted tick and tick-borne disease surveillance since 1983 (Webb et al. 1990A). Early efforts documented tick species distributions through vegetation flagging and sampling of host animals such as deer, small mammals, and lizards. Serological testing assessed pathogen exposure. These activities supported the development of identification resources for all life stages of ticks and informed public education on tick-borne disease risk (Webb et al. 1990A).

Prior to adopting PCR testing in 2009, pathogen detection relied on collaborations with universities and public health agencies. Ticks and hosts were tested for pathogens including *Borrelia*, *Babesia*, *Ehrlichia* and *Rickettsia* species (Billeter et al. 2017; Meyers et al. 1992; Barlough et al. 1997; Wikswo et al. 2008). No records were documented testing for *Francisella tularensis*. The most recent tick-borne pathogen identified in Orange County was *Rickettsia rickettsii* subsp. *californica*, detected in ticks in 2022. Overall, pathogen prevalence in Orange County ticks historically has been low, though not absent.

Human cases of tick-borne disease in Orange County are rare. Lyme disease became reportable in California in 1989, with occasional locally acquired cases documented (Meyers et al. 1992). There have also been isolated reports of spotted fever group rickettsiosis, which are thought to be caused by *Rickettsia philipi*, now reclassified as *Rickettsia rickettsii* subsp. *californica* (CDC 2010).

The OCMVCD tick program includes tick collection, host sampling, and pathogen testing. The Tick Response Plan

outlines coordinated actions in response to tick infestations, human cases, disease clusters, and pathogen detections. The current paper summarizes OCMVCD surveillance strategies, program improvements, and response protocols.

Surveillance Methods

In 2024, OCMVCD restructured its tick surveillance program to improve consistency, standardize data collection, and reduce spatial bias. Tick surveillance occurs between December and May due to operational demands associated with year-round mosquito control and surveillance. A minimum of 15 surveillance sites per season was established as a sustainable baseline effort. Additional surveillance sites are identified through staff observations, inspector communication, public service requests, and citizen science platforms such as iNaturalist, which may aid in detecting novel or invasive tick species (Foley et al., 2025). Site selection is guided by the presence of human activity to assess exposure risk, the availability of suitable tick-host interfaces such as areas with deer or other wildlife, and site accessibility and safety. Emphasis is placed on trailheads and early trail sections in high use recreational areas.

Nine sites were designated as routine surveillance locations based on historical abundance, prior pathogen detections, and site suitability (Fig. 1). These are surveyed annually to support long-term data continuity. To improve geographic coverage, Orange County was divided into three zones, with one zone receiving prioritized surveillance each year through the addition of six rotating sites. This

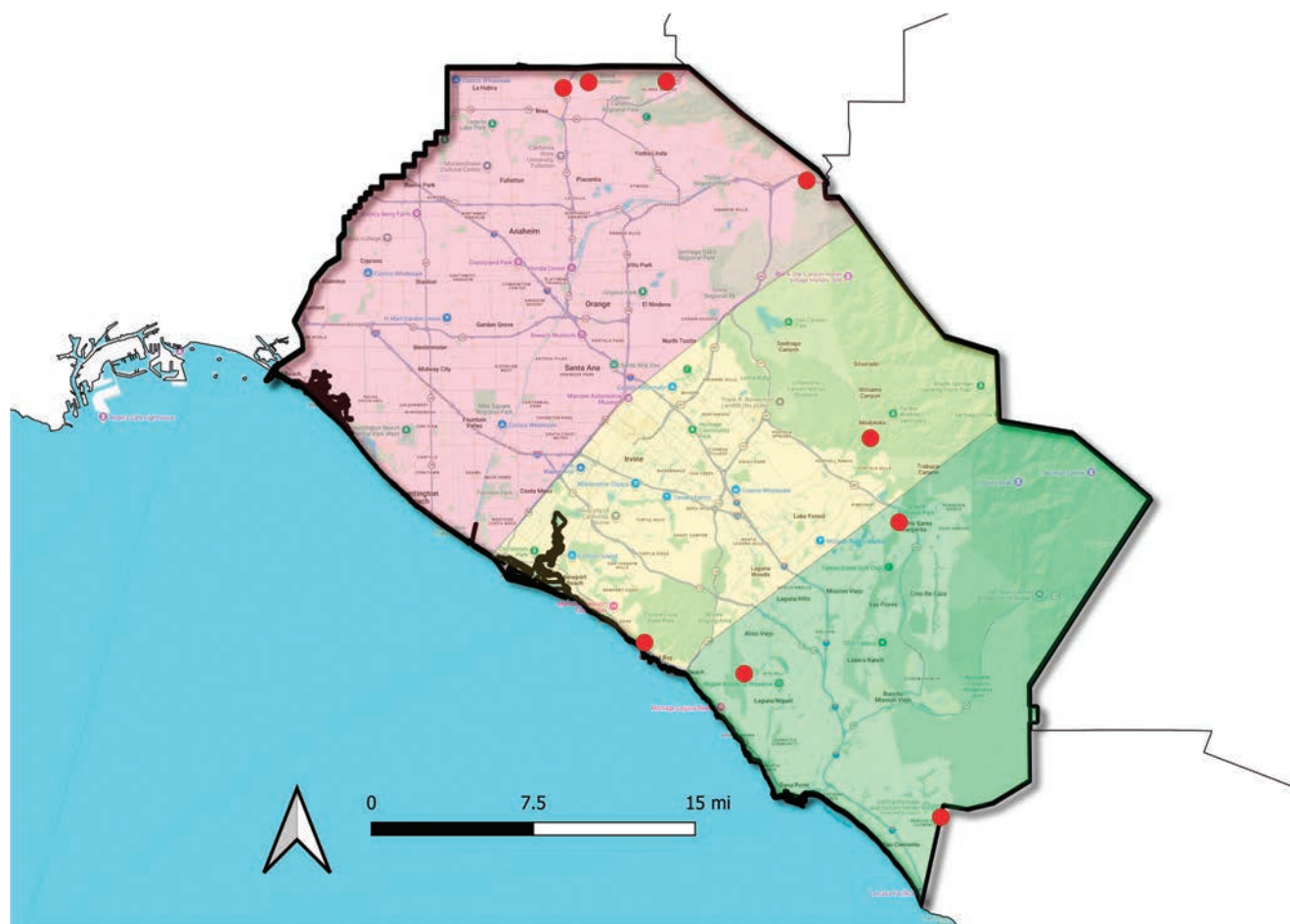


Figure 1.—Three tick surveillance zones (red, yellow, green) and nine routine tick surveillance sites (red dots) in Orange County, CA

rotational framework ensures that all regions are sampled on a three-year cycle while maintaining consistency at routine sites. Coordination with landowners and land managers occurs prior to surveillance to secure access and communicate planned activities.

Data collection was standardized through the Tick Collection Field Report (TCFR), which aligns with updated state reporting requirements. The TCFR captures key variables including date, time, location, personnel, habitat, collection method, sampling duration, and meteorological conditions such as temperature and relative humidity. This approach enables comparisons across sites and years and supports the development of effort-based metrics, such as ticks collected per hour.

Tick collection methods are selected based on local habitat and tick behavior. Flagging is the primary method used due to the predominance of coastal sage scrub habitats in the region, where dragging is less effective. Passive carbon dioxide trapping is not routinely used because local tick species exhibit less aggressive host-seeking behavior. Lizard noosing was reintroduced to improve collection of immature life stages, because flagging during winter and spring primarily yielded adult ticks. Captured lizards are inspected for ticks and other ectoparasites and released immediately after processing (Bennett and Webb 1985; Webb et al. 1990B).

Lizards most commonly processed are *Sceloporus occidentalis* and *Elgaria multicarinata*.

Collected ticks are preserved in 70% isopropanol and transported to the laboratory for identification and testing. Specimens are identified to species, sex, and life stage, then pooled (up to five individuals of the same species and sex) for pathogen testing using real-time PCR Testing protocols include a California Department of Public Health assay targeting *Borrelia burgdorferi sensu stricto*, *Borrelia americana*, and *Borrelia bissettiae*, and an in-house assay targeting *Rickettsia rickettsii* and *R. rickettsii* subsp. *californica*. Positive samples are submitted for state confirmation, and relevant stakeholders are notified.

Results

During 2024 and 2025, a total of 118 surveillance hours were conducted, resulting in the collection of 2,479 ticks. Of these, 740 ticks were tested in 2024 and 1,739 in 2025. The majority were *Dermacentor occidentalis* (88%), followed by *Ixodes pacificus* (10%), and *Dermacentor similis* (2%). One *Ixodes angustus* specimen was collected. All ticks tested negative for targeted pathogens, and no nymphs were collected while flagging during this period. Seven *Sceloporus occidentalis* lizards were captured by noosing and none were

found to carry ticks. Collection rates increased from 8.55 ticks per hour in 2024 to 24.55 ticks per hour in 2025.

Discussion

Several programmatic gaps were identified in the OCMVCD tick surveillance program, including inconsistent annual effort, seasonal limitations, non-standardized data collection, lack of comparable metrics, and regional sampling bias. Historical surveillance varied widely between years, with no established protocol guiding effort, resulting in inconsistent data and limited comparability.

Surveillance was largely restricted to the December through May mosquito off-season, which biased collections toward adult ticks and limited detection of nymphs, a life stage known to have higher pathogen prevalence. This seasonal constraint also influenced species representation, as winter and spring collections are dominated by *Dermacentor occidentalis*, whereas summer sampling would likely increase detection of other species such as *Dermacentor similis*. The absence of a standardized effort metric further limited trend analysis. Additionally, surveillance sites were historically concentrated in southern Orange County, creating regional bias and limiting representation of the County's diverse habitats, land use patterns, and microclimates. These limitations were the basis of the programmatic shifts in 2024.

Legal Authority

The California Health and Safety Code (Sections 2000–2093) provides mosquito and vector control districts with authority to conduct surveillance, prevention, and control of vectors of public health concern. Guidance from state agencies further clarifies that vector control districts are exempt from scientific collection permit requirements when conducting surveillance activities.

The collection of native reptiles is regulated under California Code of Regulations Title 14, which permits such activities under a sport fishing license (CDFW 2015; CDFW 2023). Human cases of reportable vector-borne diseases are communicated to OCMVCD through coordination with the Orange County Health Care Agency under established agreements (OCHCA 2012). District staff are trained annually in HIPAA compliance, and information sharing during investigations is conducted in accordance with these regulations.

Future Goals

The primary objective of the 2024 program restructuring was to address gaps in data collection consistency, data standardization, and spatial representation. OCMVCD will continue to evaluate program performance and refine methodologies as additional data become available.

Future improvements include incorporating the TCFR into a digital system to enhance data quality and efficiency,

expansion of lizard noosing into early summer to improve collection of immature ticks, and increased focus on pathogen detection through more representative sampling across life stages.

The District also aims to strengthen communication with landowners, public stakeholders, and partner agencies, particularly in response to elevated tick activity, pathogen detections, or human cases. Continued evaluation will be necessary to determine the long-term effectiveness of these program enhancements in improving tick surveillance in Orange County.

Acknowledgements

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A planning tool for Brown Dog Tick control and Rocky Mountain spotted fever prevention

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Introduction

The brown dog tick (BDT, *Rhipicephalus sanguineus* sensu lato) is a globally distributed ectoparasite and a vector of deadly pathogens, including *Rickettsia rickettsii*, the etiologic agent of Rocky Mountain spotted fever. Its indoor survival, host specificity to domestic dogs, and ability to complete its life cycle on a single host in a short time span makes infestations uniquely challenging to detect and control. Despite the rising incidence of infestations in California, there is no centralized resource integrating tick ecology, surveillance methods, and intervention planning. We are developing a modular educational and simulation platform to train practitioners, support academic instruction, and guide evidence-based control strategies.

Methods

The platform includes four modules. The Ecology and Behavior module explores brown dog tick biology, life cycle, and behavior, emphasizing why this species is especially challenging to detect and manage. The Surveillance and Control Protocols module provides field-ready methods, outbreak case studies from California, and decision-making guides for integrated pest management. The *Rhipicephalus sanguineus* Assessment, Prevention, Intervention, & Decision-support Simulator (RAPID) is a Shiny web application (<https://shiny.posit.co/r/getstarted/shiny-basics>). It enables users to input local infestation parameters, select interventions, and visualize projected outcomes. RAPID serves both as a planning tool for

agencies and as a teaching aid for students learning vector and disease modeling. Users can explore intervention effects across different climates, infestation sizes, and dog populations, supporting control of both brown dog ticks and RMSF. The platform is accessible at <https://shiny.pacvec.us/RAPID>. The Public Education Toolkit provides multilingual handouts and infographics to promote community engagement in early detection and outbreak response.

Conclusions

This platform equips vector control agencies and students with practical, evidence-based tools for managing brown dog tick infestations and mitigating RMSF risk. By combining training, standardized protocols, simulation-based planning, and community engagement, it bridges the gap between theory and field application.

Acknowledgements

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Surveillance and identification of *Culicoides* species in Fresno County

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Introduction

West Coast surveillance and identification of *Culicoides* populations remains a major knowledge gap. It is increasingly important to fill this gap because *Culicoides* are known vectors of disease agents. Species-level identification is crucial, considering that host and habitat preferences create specificity between diseases and the species that can transmit them. For example, *Culicoides paraensis* is the primary vector of Oropouche virus, whereas other species such as *Culicoides sonorensis* transmit bluetongue virus (McGregor et al. 2022). *Culicoides paraensis* has yet to be reported in Fresno County; however, there have been reports in states as far west as Nebraska and Colorado. This makes continued surveillance of *Culicoides* populations critical for nationwide surveillance. Understanding the abundance and distribution of local *Culicoides* populations is essential for assessing their disease transmission potential and for implementing effective prevention strategies against emerging disease threats. The objective of our research project was to gain insight into the abundance and distribution of *Culicoides* in Fresno County, California.

Methods

For the collection of *Culicoides*, modified encephalitis virus surveillance (EVS) traps were set weekly from June through October. Traps were constructed according to a protocol from the University of Florida (Burkett-Cadena 2025). Because EVS style traps were used in place of CDC light traps, the initial design lacked the mesh sleeve described by the protocol. To correct the resulting lack of airflow, two layers of mesh were added to the main collection chambers. Trap efficiency increased substantially after this modification.

Identifications were completed using dichotomous and visual keys in *Culicoides* and *Leptoconops* of the *Southwestern U.S.* (Phillips 2022). Female specimens were identified primarily based on wing patterns whereas males were identified based on both wing pattern and aedeagus morphology. A combination of dissecting and compound microscopes were used with magnifications

ranging from 20x-400x. Lower magnifications were used for whole sample sorting, whereas higher magnifications were used for observing specific structures such as the male aedeagus.

Results and Discussion

A total of 1,012 *Culicoides* specimens were collected and identified, including 964 females and 48 males. The high prevalence of female specimens is consistent with the use of CO₂ baited traps. Species composition was as follows: 978 *Culicoides sonorensis*, 17 *Culicoides crepuscularis*, 14 belonging to the subgenus *Silvicola*, 2 *Culicoides reevesi*, and 1 *Culicoides defoliarti-haematopotus*. All males were identified as *Culicoides sonorensis*, which helped to inform the female identifications of *C. sonorensis* because female *C. sonorensis* and *C. occidentalis* specimens are morphologically indistinguishable. Male specimens are identified by the presence (*C. sonorensis*) or absence (*C. occidentalis*) of spicules on the aedeagus. Additionally, *C. sonorensis* prefers highly organic soil whereas *C. occidentalis* prefers high-salinity soil. A combination of male identifications and the soil type of trapping sites gave a high degree of confidence in female *C. sonorensis* identifications.

Conclusions

Our research project succeeded in providing insight into *Culicoides* abundance and distribution within Fresno County. *Culicoides paraensis* was not detected during our survey; however, continued surveillance is important with the emerging threat of Oropouche virus. In total, 96.6% of the collected specimens were identified as *Culicoides sonorensis*, a known vector of bluetongue virus and epizootic hemorrhagic disease (McGregor et al. 2022). Fresno County is within a high-risk area for these diseases, making widespread surveillance an important next step. Additionally, this project established a protocol for *Culicoides* collection and identification at Consolidated Mosquito Abatement District. In the future, systematic surveillance of *Culicoides* populations will be necessary to better understand their distribution within Fresno County.

Acknowledgements

I would like to thank Dr. Erik Blosser for his on-site assistance with the identification process. I would also like to thank Jodi Holeman, Katherine Ramirez, and Jovana Benavides for their continued academic and professional support.

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Operation: Firestorm – Taking on RIFA

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Introduction

The Coachella Valley Mosquito and Vector Control District (District) boundary encompasses 2,400 square miles, including golf courses, parks, residential communities, and schools creating ideal urban habitats for invasive insects. Daily irrigation, along with large expanses of turfgrass and planted trees, produces humid conditions that are perfect for *Solenopsis invicta*, the red imported fire ant (RIFA). The California Department of Food and Agriculture (CDFA) discovered RIFA in the City of Palm Springs in 1998, which led to the local District's involvement in 2005. Residents of the Valley affected by RIFA infestations can now contact the District for property inspections and pest control treatments. This invasive species is prevalent in the Coachella Valley, inflicting painful stings and damaging lawns, thereby adversely affecting residents' quality of life. The District aims to manage the RIFA population by implementing a variety of control strategies. This approach aims to eliminate ants in areas where they pose the greatest risk to people, pets, and livestock. To address this issue, the District allocates resources for the management of RIFA activity. The District's objective is to maintain RIFA populations at acceptable levels where they do not become a nuisance or a public health concern. The following overview analyzes the daily control strategies and various methods used in the RIFA program.

Methods

The District's RIFA program is designed to mitigate the potential for injury and reduce the economic impact on both residents and visitors in the valley. The RIFA program is staffed by four full-time vector control technicians (VCTs) and one field supervisor, ensuring effective implementation and oversight. The RIFA team manages residential service requests. Each service request populates a 'service request form' by call center personnel with type of service, schedule date, address, contact information, phone number, email, and problem description. Next, the supervisor assigns the service request to a VCT in the RIFA program. The technician checks if there have been any previous inspections, treatments, or RIFA at this location. Then the technician reads the problem description to determine a course of action. In 2025, the RIFA team received 1,351 service requests, which varied seasonally but were consistent with the 5-year average (Figure 1). The RIFA team also conducts inspections and treatments for homeowners' associations (HOAs), golf

courses, parks and school sites. Property inspections and the application of control products are performed at scheduled intervals.

In 2025, Golf courses can participate in the RIFA maintenance bag program. A RIFA maintenance agreement contract is provided to the golf course owner or superintendent. The District takes appropriate measures to train the golf course personnel and will donate x pounds of E+ depending on the acreage of the golf course. Additionally, the District will provide the product label, Safety Data Sheet (SDS), and calibration cards. A golf course staff member who is a certified pesticide applicator must perform the treatments. The owner is required to report all pesticide use to the Riverside County Agricultural Commissioner monthly. A pre-treatment survey is done prior to any golf course application. Prior to the application, the District uses a baiting survey to determine the presence of fire ant activity. Ten baits (hot dog slices) are placed with a flag at the perimeters of the tee box, the fairway, and the green typically in the rough (30 baits per hole) of six non-consecutive holes. Both sides of hole are surveyed. After 60 minutes, the number of positive baits is recorded. Numbers are calculated as a positive percentage: either 90 or 180 baits, depending on the number of holes on the golf course. There are currently eleven (11) golf courses enrolled in the RIFA maintenance bag program.

Depending on the extent of the infestation, pesticides are applied by 'hand', backpack, or golf cart with a Herd Seed Spreader. In addition, the District operation and laboratory teams collaborated with Central Life Sciences to evaluate the effectiveness of drone applications. Prior to the drone application, a survey was conducted in a 40-acre field. Quadrants A, B, C, & D each covered an area of 1 acre. Thirty baits were dispersed per quadrant, for a total of 120 baits. A scoring system was given to the approximate number of ants on the baits (Figure 2). The number of ants on hot dogs were measured one week before and three weeks after the treatment.

Results and Discussion

In 2025 the RIFA team did 1,994 broadcast treatments of Extinguish Plus® (E+) Insecticide Fire Ant Bait (active ingredients: 0.365% hydramethylnon and 0.250% S-methoprene), Advion® Insecticide Fire Ant Bait (active ingredient: 0.045% indoxacarb), and Siesta® Insecticide Fire Ant Bait (active ingredient: 0.063%)

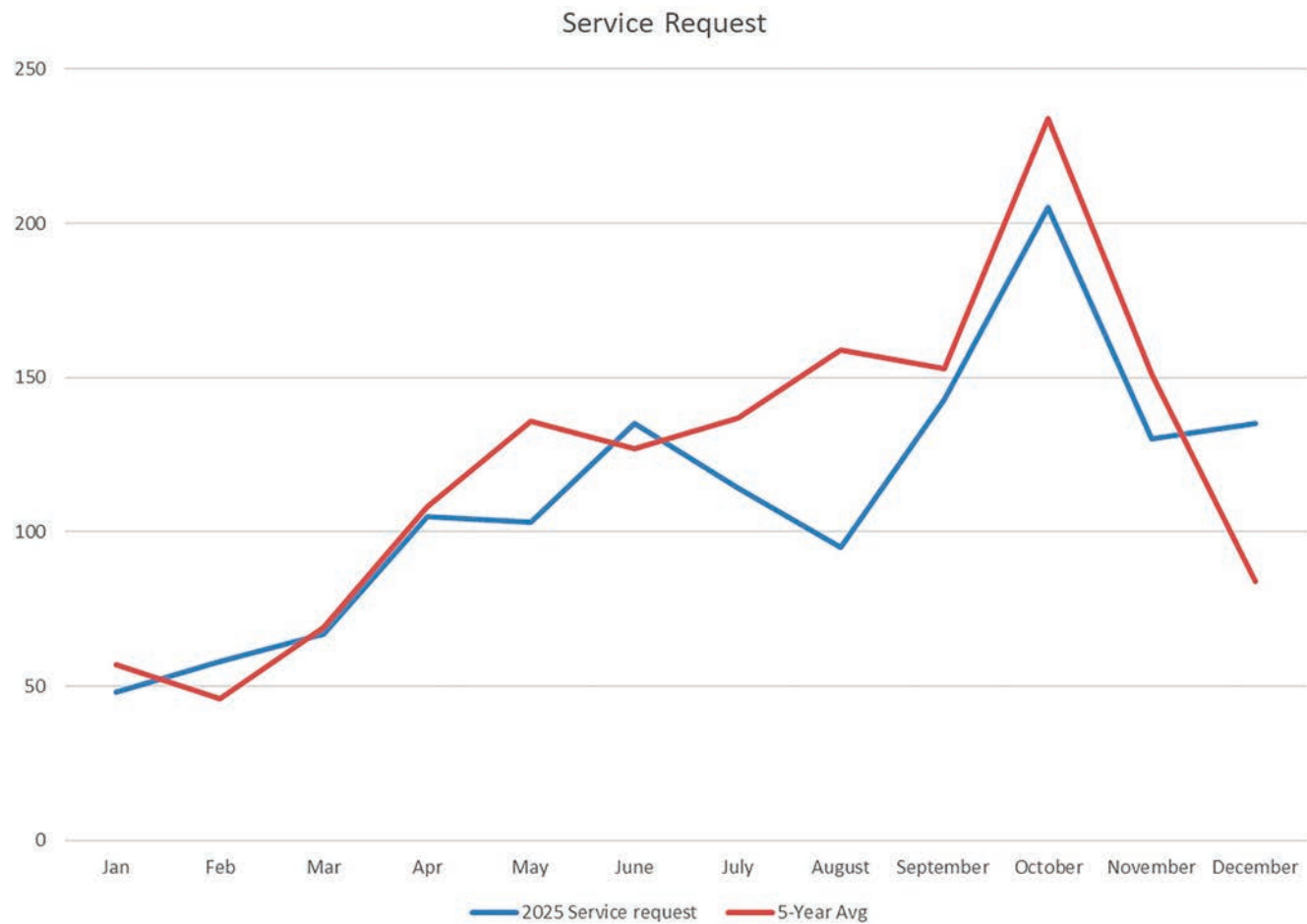


Figure 1.—Service Requests in 2025 compared to a 5-year average.



Figure 2.—Survey area prior to drone treatment and number of ants scoring table.

Property	2024 Treatment Count	2024 Total Acres	2025 Treatment Count	2025 Total Acres
HOAs	428	6,898.79	396	4,107.31
Golf Courses	55	4,063.04	36	1,902.78
Parks	228	2,217.36	226	1,891.86
Residential	1049	483.62	1167	364.63
Schools	127	1,919.75	169	1,616.21
Grand Total	1,887	15,482.56	1,994	9,882.79

Figure 3.—2024 and 2025 RIFA treatment count and acres table.

using a handheld spreader, Herd Seed Spreader, and Maruyama backpack use to apply insecticide at 1.5 lbs. per acre. Applications covered a total of 9,882.79 acres. In 2025, VCTs in the RIFA program conducted more treatments, but the total number of acres treated was lower compared to 2024. This decrease in treated acres was due, in part, to golf course participation in RIFA maintenance bag program, and the involvement of a golf course applicator in administering the treatments. (Figure 3). E+ showed reduced RIFA activity on 8 of the 11 golf courses enrolled in the maintenance bag program in which the District donated 765 lbs. of control product in 2025. Following the drone pilot treatment with E+, a reduction in the number of ants at all sites (quadrants) was noted (Figure 4).

Conclusions

The District's RIFA program is both dynamic and adaptable, effectively responding to the evolving challenges of fire ant control within the Coachella Valley. This initiative

Row Labels	Average of before treatment	Average of after treatment
A	6.2	0.27
B	5.13	0.77
C	5.4	0.13
D	6.2	0.17
Grand Total	5.73	0.33

Figure 4.—Infestation rate table for golf courses enrolled in the RIFA maintenance bag program.

emphasizes selecting suitable control products and application equipment, thereby significantly enhancing operational efficiency and reducing the abundance of fire ants. Such a proactive strategy aims to improve the community's overall quality of life by alleviating concerns about disruptions or health risks associated with RIFA. We anticipate continued growth in the RIFA program, particularly with the integration of innovative control strategies, such as drone deployment for fire ant treatments. The next phase of our efforts involves incorporating scheduling tools and the functionality of the RIFA maintenance bag program to bolster our fire ant control initiatives further to protect public health.

Acknowledgments

I thank the team involved in the Red Imported Fire Ant (RIFA) program for their ongoing control efforts. I would also like to acknowledge the Lead Vector Control Technicians for their significant contributions to the RIFA maintenance bag program. Lastly, I express my appreciation to the unmanned aerial system (UAS) team, Central Life Sciences, the District laboratory, and the operations department for their dedication to advancing the RIFA program.

Get them outside: Teaching IPM by bringing schools into wetlands

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Abstract

In Minnesota we have had success partnering with schools to teach IPM by getting students out of the classroom and into a local pond or wetland where mosquito larvae may be present. This presentation shared some examples of this style of presentation and some of the key successes that have been achieved. In the presentation, we included:

- Using GIS to identify mosquito habitat near a school
 - School outreach to set up presentations
 - The tools used for outdoor learning
 - Learning objectives for students of different ages
 - Recent examples of this style of presentation
-

Deputizing our youth to fight vector-borne illness

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Abstract

Everyday we fight a war we know we can't win, but we do it to save as many people as we can from vector-borne illnesses. We treat as much as we can sample in one day and try again the next. Our project focuses on empowering our children to be Mosquito Deputies. Our kits are basically mini sample kits with location form, pre-addressed return envelopes for their samples, and instructions on what to do if they find larva. Essentially, they would be patrolling their own living space and eliminating sources at the same time. We receive and test the samples and send the child a thank you card with findings and encouragement to keep up the great job. The larger this project expands the more help we receive reducing mosquito populations and disease. Kids that dump over mosquito larval sources will become adults that do the same and teach their children to continue the fight. This project could have long and far-reaching benefits.

Engaging local college students in vector-borne disease research at agencies across the region through the PacVec summer internship program

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Abstract

In 2025, PacVec partnered with 9 agencies across 5 states to host 12 summer interns. These internships provided career development opportunities for local students or recent graduates interested in public-health-relevant research on vector-borne diseases. As a part of the experience, interns gained exposure to multiple aspects of the partner agency and conducted a project supervised by a designated agency staff member. The interns joined the PacVec trainee network to network and build skills, and the interns each presented their research at a conference this year. This presentation outlined the internship program, shared the outcomes of the 2025 program, and discussed plans for the 2026 program.

Creating an efficient interactive school program

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Abstract

School outreach comprises an important part of the San Diego County Vector Control Program (VCP) Integrated Vector Management strategy. The county's 42 school districts with over 1000 schools, 470,000 K-12 students, parents, and families provide an opportunity to reach a widespread audience through in-person school presentations. However, in 2020 due to COVID restrictions, VCP developed the Let's Learn About Mosquitoes Program (LLAMP) to provide virtual presentations that still included hands on activities and could be done with minimal staff. The LLAMP program has three parts: 1) an introductory class activity led by the teacher, 2) a take home activity, and 3) a week later, a classroom presentation given by VCP staff. To make the program easier to promote to teachers, the lesson plans were developed to meet Next Generation Science Standards for 4th-5th graders. For the teacher led portion, an activity box containing a teacher's guide, sample slides for an in-class activity, magnifying glasses, and student laboratory notebooks, is delivered to the teacher. The teacher gives the lesson to their students and then sends a take-home assignment with them to find, record, and remove water sources around their homes. One week later, VCP staff give a final presentation to the students, take questions, and evaluate their learning.

This school outreach education structure has resulted in distinct benefits to the students, teachers, and VCP staff. Staff have observed that having the teacher complete the in-class and at-home activities prior to the VCP presentation has led to higher engagement between students and VCP staff than when VCP conducted school presentations first. The prior school programs also required at least two VCP staff to be present, an hour for the presentations, and time for set-up and take-down. The LLAMP now only requires one staff member and 30 min for the presentation with time for questions. Because the teacher controls the hands-on activity, fewer staff are required to manage the classroom. The reduction in time and staff required has enabled the VCP outreach team to do multiple presentations on the same day. Additionally, because the hands-on activity is completed before the presentation, classes can be grouped together for an assembly presentation. This combination makes the LLAMP more cost efficient for the VCP, able to reach more students, and still provide an interactive program. Over 800 quizzes given to students before and after the LLAMP program demonstrated an increase score average of 24% (70% average pre-program test score; 94% post average pre-program test score) which showed that students increased their mosquito knowledge even with the shorter classroom program.

Over the last four years, the LLAMP has more than doubled the average number of yearly school presentations, with an average of 16 schools and 32 presentations per year, compared to 8 schools and 11 presentations with the previous program. The program is in its 5th year, with several teachers and schools returning for multiple years. The success of the program has also allowed the VCP to expand to include 2nd and 3rd grade, middle and high school programs, and helped the team develop new programs for scouting groups and libraries. The set-up makes it easier to give more presentations and visit more schools, even with less staff and time. This program is consistent yet flexible and has received positive responses from teachers and students. It can be replicated by other programs to create their own interactive, effective, and efficient school program.

Surveillance for mosquito-borne virus activity in California, 2025

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Abstract

In 2025, the California surveillance program for mosquito-borne encephalitis virus activity tested humans, horses, dead birds, mosquitoes, and sentinel chickens. West Nile virus (WNV) activity was reported from 36 of 58 counties in California, and St. Louis encephalitis virus (SLEV) activity was reported from 3 counties. A total of 116 human WNV cases were reported, and non-human WNV activity was detected among horses, dead birds, mosquitoes, and sentinel chickens. No cases of SLEV disease were identified, although enzootic SLEV activity was detected in mosquitoes from three counties. In addition, a total of seven locally acquired cases of dengue were identified from two counties.

Introduction

The California Arbovirus Surveillance program is a cooperative effort among the California Department of Public Health (CDPH), the University of California Davis Arbovirus Research and Training (DART) laboratory, the Mosquito and Vector Control Association of California (MVCAC), local mosquito abatement and vector control agencies, county and local public health departments, and physicians and veterinarians throughout California. Additional local, state, and federal agencies collaborated on, and contributed to, the West Nile virus (WNV) component of the arbovirus surveillance program.

In 2025, the surveillance program included the following:

- (1) Diagnostic testing of specimens from human patients who exhibited symptoms compatible with WNV disease as well as blood bank and organ donor screening for WNV infection.
- (2) Monitoring mosquito abundance and testing mosquitoes for the presence of WNV, St. Louis encephalitis virus (SLEV), western equine encephalitis virus (WEEV), and other arboviruses as appropriate.
- (3) Serological monitoring of sentinel chickens for WNV, SLEV, and WEEV antibodies.
- (4) Reporting and testing of dead birds for WNV.
- (5) Weekly reporting of arbovirus test results to ArboNET, the national arbovirus surveillance system.

- (6) Weekly reporting of arbovirus activity in the CDPH Arbovirus Surveillance Bulletin and on the California WNV website (<https://westnile.ca.gov>).
- (7) Data management and reporting of non-human data through the CalSurv Gateway, the California arbovirus surveillance system.

West Nile virus activity was reported from 36 (62%) of 58 counties in California (Table 1), while SLEV activity was reported from 3 (5%) counties (Table 2).

Human Disease Surveillance

Serological testing of human specimens for WNV and other arboviruses was conducted by local public health laboratories, commercial laboratories, and the CDPH Viral and Rickettsial Disease Laboratory (VRDL). Laboratories tested for WNV antibodies using an IgM enzyme immunoassay (EIA) and/or an IgM immunofluorescence assay (IFA). Specimens from case-patients with early season onset or from counties with enzootic SLEV activity received confirmatory testing using plaque reduction neutralization tests (PRNT) at VRDL. Additional WNV infections were identified through screening performed by blood centers.

In 2025, a total of 116 symptomatic and 12 asymptomatic infections with WNV were reported (Tables 1 and 3). Of the 116 symptomatic cases, 103 (89%) were classified as West Nile neuroinvasive disease (WNND) (e.g., encephalitis, meningitis, acute flaccid paralysis,

Table 1.—West Nile virus activity in California by county, 2025. Humans include asymptomatic infections detected through blood bank and organ donor screening.

County	Humans	Horses	Dead birds	Mosquito pools	Sentinel chickens
Alameda	0	0	6	0	0
Alpine	0	0	NT	NT	NT
Amador	0	0	NT	NT	NT
Butte	4	0	0	28	14
Calaveras	0	0	NT	NT	NT
Colusa	1	0	1	1	1
Contra Costa	1	1	3	3	0
Del Norte	0	0	NT	NT	NT
El Dorado	0	0	0	NT	NT
Fresno	17	0	11	132	NT
Glenn	1	0	NT	0	NT
Humboldt	0	0	NT	NT	NT
Imperial	0	0	NT	1	NT
Inyo	0	0	NT	1	NT
Kern	21	0	1	129	NT
Kings	9	0	NT	129	NT
Lake	0	0	1	5	6
Lassen	0	0	NT	NT	NT
Los Angeles	24	0	5	200	18
Madera	3	0	0	264	NT
Marin	0	0	0	0	NT
Mariposa	0	0	NT	NT	NT
Mendocino	0	0	NT	NT	NT
Merced	3	0	1	42	NT
Modoc	0	0	NT	NT	NT
Mono	0	0	NT	0	NT
Monterey	1	0	0	NT	NT
Napa	0	0	0	0	NT
Nevada	1	0	0	NT	0
Orange	2	0	4	186	NT
Placer	6	0	8	145	NT
Plumas	0	0	NT	NT	NT
Riverside	1	0	0	15	NT
Sacramento	2	1	27	67	NT
San Benito	0	0	0	0	0
San Bernardino	0	0	0	6	NT
San Diego	0	0	33	181	NT
San Francisco	0	0	0	0	NT
San Joaquin	7	0	7	139	NT
San Luis Obispo	0	0	0	0	NT
San Mateo	0	0	0	0	NT
Santa Barbara	0	1	0	0	NT
Santa Clara	2	0	26	3	NT
Santa Cruz	0	0	0	0	NT
Shasta	0	0	0	2	0
Sierra	0	0	NT	NT	NT
Siskiyou	1	0	NT	NT	NT
Solano	1	0	3	28	2
Sonoma	0	0	1	0	NT
Stanislaus	2	1	1	196	NT
Sutter	2	0	1	12	11
Tehama	0	0	NT	NT	0
Trinity	0	0	NT	NT	NT
Tulare	13	0	8	759	9
Tuolumne	2	0	NT	NT	NT
Ventura	0	0	1	0	0
Yolo	1	1	5	77	NT
Yuba	0	0	0	8	4
State Totals	128	5	154	2,759	65

NT = None tested.

Table 2.—St. Louis encephalitis virus activity in California by county, 2025.

County	Humans	Mosquito pools ¹	Sentinel chickens
Fresno	0	1	NT
Imperial	0	8	NT
Riverside	0	96	NT
State Totals	0	105	0

¹ Positive mosquito pools included *Culex quinquefasciatus* (13) and *Culex tarsalis* (92). NT = None tested.

or other neurologic dysfunction) and 13 (11%) were classified as non-neuroinvasive disease; 11 (10%) cases were fatal. Cases were residents of 25 counties, and 78 (67%) were male. In 2025, WNV disease incidence in California was 0.29 cases per 100,000 persons. Incidence was highest (5.84 cases per 100,000 persons) in Kings County, whereas Los Angeles County reported the most cases (20, 18% of total) (Figure 1, Table 3). The median age of those with WNND was 65 years (range, 32 to 88 years), and among cases with non-neuroinvasive disease the median age was 59 years (range, 37 to 69 years). The median age of the 11 WNV-associated fatalities was 73 years (range, 36 to 88 years). Dates of symptom onset ranged from June 6 to November 23, with the peak occurring during epidemiological week 36 (August 31 – September 6), when 16 (14%) symptomatic infections occurred.

In addition to human cases of WNV, seven locally acquired cases of dengue were identified in Imperial (1) and Los Angeles (6) counties. This is the third consecutive year that locally acquired cases of dengue, a virus transmitted by invasive *Aedes* mosquitoes, have been identified in California.

Mosquito Surveillance

In 2025, mosquito testing was performed at DART and 15 local mosquito and vector control agencies. A total of 1,466,666 mosquitoes (53,780 pools) collected in 40 counties were tested by a real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) for SLEV, WEEV, and/or WNV viral RNA (Table 4). *Aedes aegypti* mosquitoes also were tested for chikungunya, dengue, and Zika viruses at DART by a separate triplex RT-qPCR.

West Nile virus was detected in 2,759 mosquito pools from 27 counties (Tables 1 and 4), and the statewide annual minimum infection rate (MIR-defined as the minimum number of infected female mosquitoes per 1,000 tested) of WNV in all mosquitoes tested was 1.9. During California's peak transmission period (July – September) the statewide MIR in *Culex* mosquitoes was 3.1 and 6 counties reported MIRs greater than 5.0, the epidemic threshold value defined in the statewide response plan (Figures 2 and 5) (California Department of Public Health, 2025). West Nile virus was detected

Table 3.—Reported West Nile virus human cases by county of residence and year, California, 2016–2025.

County	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2025 incidence per 100,000 person-years	10-year incidence per 100,000 person-years
Alameda	0	1	0	1	0	0	1	1	0	0	0.00	0.02
Alpine	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Amador	1	0	1	1	0	0	0	0	0	0	0.00	0.76
Butte	21	4	12	5	4	13	3	18	11	4	1.93	4.58
Calaveras	0	0	0	0	0	0	0	1	1	0	0.00	0.45
Colusa	2	0	0	1	0	0	1	1	1	1	4.54	3.18
Contra Costa	4	4	4	1	4	2	1	10	4	1	0.09	0.30
Del Norte	0	0	0	0	0	0	0	0	0	0	0.00	0.00
El Dorado	1	0	0	0	1	1	0	3	0	0	0.00	0.31
Fresno	14	13	14	51	10	14	30	23	17	15	1.45	1.94
Glenn	6	0	2	0	1	2	1	4	1	1	3.40	6.13
Humboldt	0	0	1	0	0	0	0	0	0	0	0.00	0.07
Imperial	0	3	0	3	1	0	0	0	0	0	0.00	0.38
Inyo	0	4	0	0	0	0	0	0	0	0	0.00	2.13
Kern	17	30	13	28	8	8	22	16	12	19	2.09	1.87
Kings	8	5	0	3	2	8	7	9	6	9	5.84	3.70
Lake	1	0	1	0	2	0	0	6	0	0	0.00	1.49
Lassen	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Los Angeles	151	277	43	31	90	16	61	62	23	20	0.20	0.78
Madera	6	2	4	3	6	3	3	9	0	1	0.63	2.28
Marin	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Mariposa	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Mendocino	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Merced	0	10	2	10	12	6	7	8	3	2	0.68	2.05
Modoc	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Mono	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Monterey	1	0	1	0	0	0	0	2	0	1	0.23	0.11
Napa	0	0	1	0	0	0	0	0	0	0	0.00	0.07
Nevada	0	0	1	0	0	0	0	1	0	1	1.00	0.30
Orange	32	33	9	5	17	3	9	6	1	2	0.06	0.37
Placer	7	0	9	1	2	2	2	6	0	6	1.42	0.83
Plumas	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Riverside	11	32	15	12	10	3	0	20	6	1	0.04	0.44
Sacramento	25	6	15	4	7	6	5	53	7	2	0.12	0.81
San Benito	0	0	0	0	0	0	0	0	0	0	0.00	0.00
San Bernardino	8	57	9	7	3	1	4	28	5	0	0.00	0.55
San Diego	20	2	2	3	1	3	3	0	2	0	0.00	0.11
San Francisco	0	1	0	0	0	1	0	0	0	0	0.00	0.02
San Joaquin	13	14	14	7	2	7	4	15	2	7	0.88	1.05
San Luis Obispo	0	0	0	2	0	2	0	2	0	0	0.00	0.21
San Mateo	0	0	0	0	0	1	1	3	0	0	0.00	0.07
Santa Barbara	0	0	0	0	0	2	0	0	0	0	0.00	0.04
Santa Clara	1	0	1	1	0	3	1	3	4	2	0.10	0.08
Santa Cruz	0	0	0	0	0	1	0	0	0	0	0.00	0.04
Shasta	1	1	1	0	2	3	1	6	1	0	0.00	0.28
Sierra	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Siskiyou	0	0	0	0	0	0	0	0	0	1	2.31	0.23
Solano	4	1	0	1	1	2	2	6	0	1	0.22	0.40
Sonoma	0	0	0	0	0	0	0	1	0	0	0.00	0.02
Stanislaus	26	28	15	16	35	5	15	33	5	2	0.36	3.24
Sutter	12	3	1	1	1	0	1	7	1	2	1.99	2.89
Tehama	5	2	2	0	2	0	3	0	1	0	0.00	2.31
Trinity	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Tulare	10	12	8	24	7	8	15	25	4	12	2.51	2.57
Tuolumne	0	0	1	0	0	0	0	0	0	2	3.68	0.55
Ventura	7	1	2	2	0	0	0	1	0	0	0.00	0.16
Yolo	16	6	11	1	4	3	3	39	9	1	0.44	4.13
Yuba	11	1	2	0	0	0	1	5	4	0	0.00	2.87
Total WNV Cases	442	553	217	225	235	129	207	433	131	116	0.29	0.68
Asymptomatic Infections	41	47	26	18	28	19	14	40	20	12		
Total WNV infections	483	600	243	243	263	148	221	473	151	128		

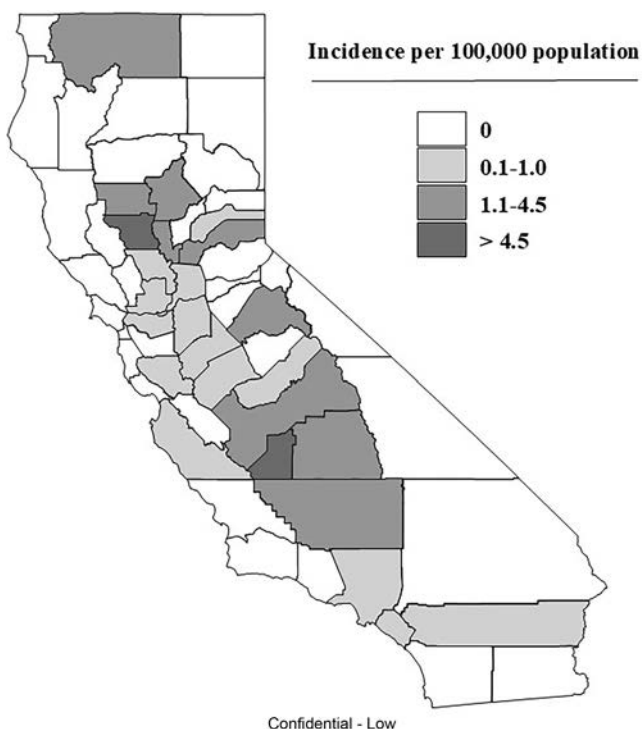


Figure 1.—Incidence of human cases of West Nile virus by county in California, 2025.

from one *Aedes* species (*Ae. aegypti*) and five *Culex* species (*Cx. erythrothorax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, and *Cx. tarsalis*) (Table 5). WNV positive pools were collected from April 25 to December 24, with the peak occurring during epidemiological week 35 (August 24 – August 30).

SLEV was detected in 105 mosquito pools from 3 counties (Table 2). Statewide, the SLEV MIR in all mosquitoes tested was 0.07; the MIR was highest (1.9) in Imperial County. St. Louis encephalitis virus was identified from two *Culex* species (*Cx. quinquefasciatus* and *Cx. tarsalis*) collected from June 17 to October 7.

In addition, a total of 32,307 *Ae. aegypti* were tested for chikungunya, dengue, and/or Zika viruses; one *Ae. aegypti* pool from Los Angeles County tested positive for dengue virus.

Chicken Serosurveillance

In 2025, 17 local mosquito and vector control agencies in 15 counties maintained 57 sentinel chicken flocks (Table 4). Blood samples were collected from chickens every other week and tested for antibodies to WNV, SLEV, and WEEV by an EIA at the CDPH Vector-Borne Disease Section and one local agency. Presumptive positive samples were confirmed by IFA or western blot. Of 4,202 chicken blood samples tested from 357 chickens, 65 (18%) seroconversions to WNV were detected among 23 flocks in 8 counties (Tables 1 and 4). Seroconversions to WNV occurred between July 22 and November 11, with the peak occurring during

epidemiological week 36 (August 31 – September 6). No SLEV seroconversions were detected.

Dead Bird Surveillance

In 2025, the WNV and Dead Bird Call Center and website received a total of 7,737 dead bird reports from 54 counties (Table 6). Tissue samples from dead bird carcasses or oral swabs transferred to RNA preservation cards (Fortius Bio, San Diego, CA) were tested by RT-qPCR by the DART laboratory and 15 local agencies. Of the 1,908 bird carcasses that were deemed suitable for testing, WNV was detected in 154 (8%) carcasses from 21 counties (Tables 1 and 6). Twenty-two different bird species tested positive for WNV: 49% were American crows, 14% were California scrub-jays, 8% were other corvids, and 29% were non-corvid or unknown species. Positive birds were detected from March 19 to November 12, with the peak occurring during epidemiological week 33 (August 10 – August 16).

Horses

Serum or brain tissue specimens from horses displaying neurological symptoms were tested for WNV at the California Animal Health and Food Safety Laboratory. In 2025, WNV infection was confirmed in 5 horses from 5 counties (Table 1); one (20%) fatality was reported.

Discussion

In 2025, 36 (62%) of 58 counties reported WNV activity. A total of 116 symptomatic human cases were reported from 25 counties, which was the lowest number of cases reported in California since 2010 (Figure 3) and lower than the 5-year average. Los Angeles County reported the most cases ($n = 20$), but the incidence was highest in Kings County (Table 3). Non-human WNV activity was reported from 31 counties and all environmental indicators except for mosquitoes were lower than the previous year. The number of WNV positive dead birds was lower than the number reported in 2024 and lower than the five-year average. In contrast, the number of WNV positive mosquito pools was higher than the number reported in 2024, but it was still lower than the five-year average. The percentage of WNV positive sentinel chicken flocks was also lower compared to previous years (Figures 4, 5 and 6; California Department of Public Health). Notably, the numbers of WNV equine cases and positive dead birds were the lowest observed since WNV emerged in CA in 2003. Surveillance results documented WNV activity throughout most of the year, but the vast majority of detections occurred from June through October, with peak activity occurring in August.

Table 4.—Results of mosquito and sentinel chicken testing for West Nile virus by county, California, 2025.

County	No. mosquitoes	No. mosquito pools	WNV + pools	No. flocks	No. chickens	No. WNV + flocks	WNV + sera
Alameda	33,766	1,230	0	1	12	0	0
Butte	21,904	475	28	7	43	6	14
Colusa	500	10	1	1	10	1	1
Contra Costa	9,690	313	3	4	20	0	0
Fresno	37,034	1,076	132				
Glenn	305	7	0				
Imperial	4,202	281	1				
Inyo	1,258	27	1				
Kern	28,751	858	129				
Kings	15,852	381	129				
Lake	16,183	556	5	2	12	2	6
Los Angeles	185,110	4,544	200	17	88	8	18
Madera	34,755	934	264				
Marin	2,365	120	0				
Merced	26,673	823	42				
Mono	28	1	0				
Napa	3,789	183	0				
Nevada				4	16	0	0
Orange	113,972	5,338	186				
Placer	39,169	2,519	145				
Riverside	259,893	7,857	15				
Sacramento	39,608	3,627	67				
San Benito	207	99	0	1	9	0	0
San Bernardino	48,817	1,854	6				
San Diego	75,038	2,740	181				
San Francisco	72	2	0				
San Joaquin	85,199	2,559	139				
San Luis Obispo	1,339	34	0				
San Mateo	5,120	244	0				
Santa Barbara	2,362	152	0				
Santa Clara	10,702	2,163	3				
Santa Cruz	1,397	117	0				
Shasta	14,998	518	2	3	20	0	0
Solano	47,694	1,297	28	3	15	1	2
Sonoma	15,070	622	0				
Stanislaus	59,052	1,658	196				
Sutter	12,318	335	12	5	33	3	11
Tehama				3	29	0	0
Tulare	148,960	5,689	759	1	10	1	9
Ventura	1,580	39	0	3	26	0	0
Yolo	53,422	2,232	77				
Yuba	8,512	266	8	2	14	1	4
Total	1,466,666	53,780	2,759	57	357	23	65

Following the re-emergence of SLEV in California in 2015, SLEV has continued to co-circulate with WNV in many areas of the state, complicating human diagnostics for these two closely related flaviviruses. Outreach to local health departments was conducted in areas with enzootic detections of SLEV and medical providers were encouraged to include SLEV testing for suspect WNV cases. However, in 2025, no human cases of SLEV were identified, marking the second consecutive year since its reemergence in 2015 that no cases were reported. A total of 105 SLEV positive mosquito pools were reported from 3 counties (Table 2), which is the lowest number of counties with reported SLEV activity since it reemerged. No SLEV chicken seroconversions were identified (Table 2). All environmental indicators of SLEV were low, supporting the absence of any identified human

cases. The lack of SLEV cases for two consecutive years since the reemergence of SLEV contrasts with the highest reported human case counts observed in 2023 and underscores the dynamic nature of environmental arboviral transmission.

Although WEEV has not been detected in California since 2007, routine testing of mosquitoes and sentinel chickens for WEEV has continued in the event this historically endemic arbovirus reemerges. In 2025, 7 locally acquired cases of dengue were reported. This marks the third consecutive year that locally acquired dengue was reported. Many of the collected invasive *Aedes* mosquitoes, *Ae. aegypti* and *Ae. albopictus*, continued to be tested for chikungunya, dengue, and Zika viruses. For the first time in California, one *Ae. aegypti* mosquito pool tested positive for dengue virus

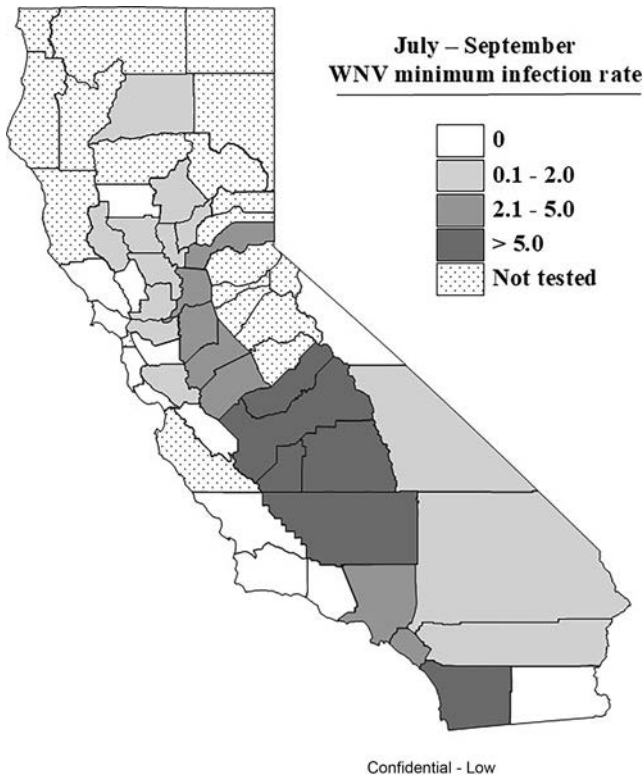


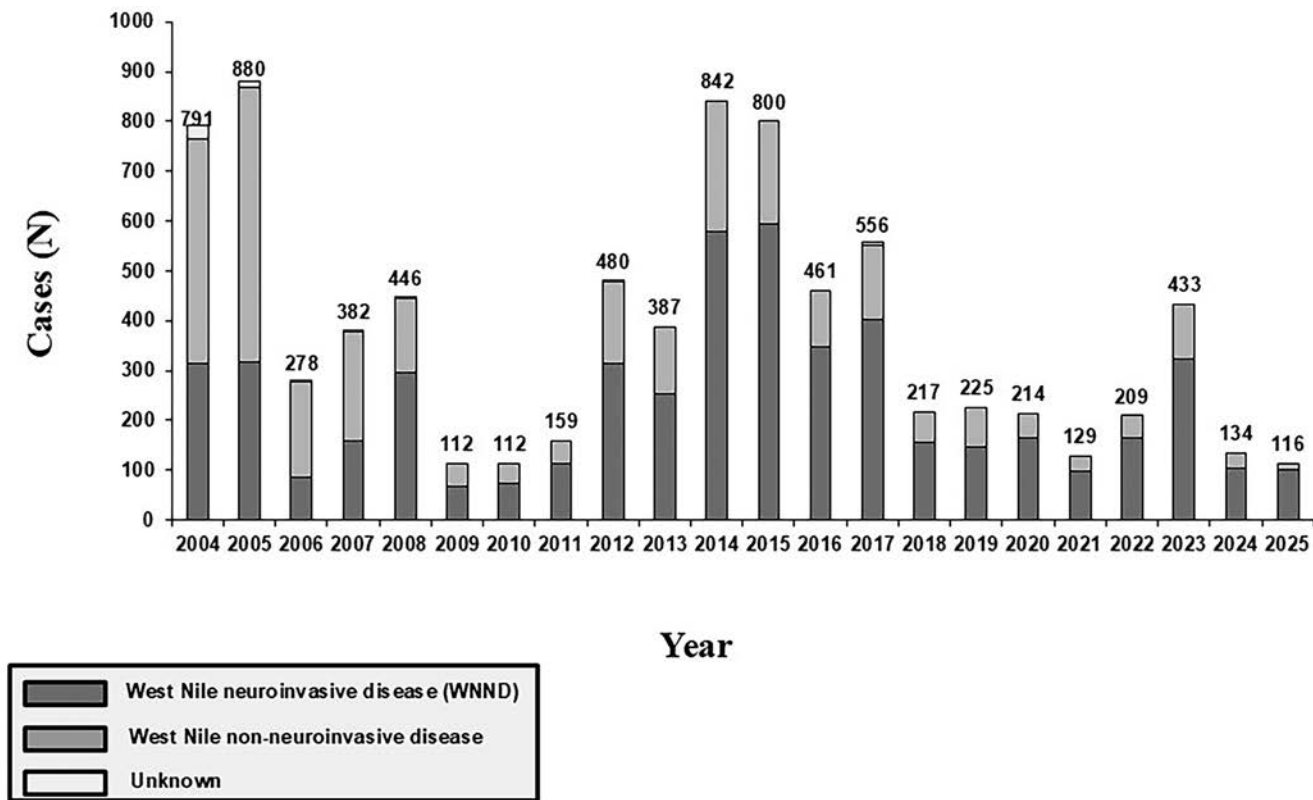
Figure 2.—West Nile virus minimum infection rate in *Culex* mosquitoes, by county, California, July – September, 2025. Minimum infection rate is defined as the minimum number of infected female mosquitoes per 1,000 tested.

Table 5.—Mosquito species tested for West Nile virus, California, 2025.

	No. mosquito pools	No. mosquitoes	WNV + pools	MIR
<i>Culex</i> species				
<i>Cx. erythrothorax</i>	2,483	146,806	2	0.0
<i>Cx. pipiens</i>	8,849	145,303	285	2.0
<i>Cx. quinquefasciatus</i>	21,076	581,979	1,369	2.4
<i>Cx. restuans</i>	7	7	0	0.0
<i>Cx. stigmatosoma</i>	1,318	16,893	65	3.8
<i>Cx. tarsalis</i>	18,192	563,785	1,036	1.8
<i>Cx. territans</i>	2	7	0	0.0
<i>Cx. thriambus</i>	115	273	0	0.0
<i>Culex</i> species	2	65	0	0.0
All <i>Culex</i>	52,044	1,455,118	2,757	1.9
<i>Aedes</i> species				
<i>Ae. aegypti</i>	1,441	4,833	2	0.4
<i>Ae. melanimon</i>	98	4,006	0	0.0
<i>Ae. notoscriptus</i>	12	65	0	0.0
<i>Ae. squamiger</i>	1	50	0	0.0
All <i>Aedes</i>	1,552	8,954	2	0.2
Other species				
<i>Anopheles hermsi</i>	1	1	0	0.0
<i>Culicidae</i>	2	13	0	0.0
<i>Culiseta incidens</i>	161	2,339	0	0.0
<i>Culiseta inornata</i>	7	40	0	0.0
<i>Culiseta particeps</i>	7	195	0	0.0
Unknown	6	6	0	0.0
All other	184	2,594	0	0.0

Table 6.—Dead birds reported, tested, and positive for West Nile virus, California, 2025.

County	Reported	Tested	WNV +	Percent
Alameda	563	225	6	3
Alpine	0	0	0	0
Amador	2	0	0	0
Butte	62	13	0	0
Calaveras	8	0	0	0
Colusa	13	4	1	25
Contra Costa	515	53	3	6
Del Norte	0	0	0	0
El Dorado	73	17	0	0
Fresno	160	17	11	65
Glenn	7	0	0	0
Humboldt	17	0	0	0
Imperial	3	0	0	0
Inyo	1	0	0	0
Kern	45	4	1	25
Kings	17	0	0	0
Lake	20	10	1	10
Lassen	1	0	0	0
Los Angeles	781	98	5	5
Madera	16	3	0	0
Marin	88	4	0	0
Mariposa	1	0	0	0
Mendocino	15	0	0	0
Merced	48	4	1	25
Modoc	0	0	0	0
Mono	1	0	0	0
Monterey	38	5	0	0
Napa	30	6	0	0
Nevada	32	4	0	0
Orange	443	157	4	3
Placer	228	83	8	10
Plumas	3	0	0	0
Riverside	177	48	0	0
Sacramento	804	298	27	9
San Benito	4	1	0	0
San Bernardino	138	31	0	0
San Diego	467	175	33	19
San Francisco	105	13	0	0
San Joaquin	215	49	7	14
San Luis Obispo	63	11	0	0
San Mateo	578	180	0	0
Santa Barbara	51	18	0	0
Santa Clara	805	172	26	15
Santa Cruz	108	33	0	0
Shasta	17	6	0	0
Sierra	1	0	0	0
Siskiyou	3	0	0	0
Solano	150	33	3	9
Sonoma	157	16	1	6
Stanislaus	161	13	1	8
Sutter	27	8	1	13
Tehama	14	0	0	0
Trinity	0	0	0	0
Tulare	73	20	8	40
Tuolumne	5	0	0	0
Ventura	132	22	1	5
Yolo	222	47	5	11
Yuba	29	7	0	0
Totals	7,737	1,908	154	8



Confidential - Low

Figure 3.—Human cases of West Nile virus in California, by year, 2004–2025.

and was collected during the investigation of a locally acquired dengue case.

Conclusions

Environmental activity of WNV and SLEV was lower in 2025 compared to recent years. WNV remains the greatest vector-borne disease threat in California, with

over 8,000 cases and more than 300 fatalities reported since its initial detection in 2003. Human cases of WNV were the lowest reported since 2010, and no human cases of SLEV were reported. Local transmission of dengue virus was observed for the third year, highlighting the importance for ongoing surveillance and awareness of potential human disease risk. Environmental detections of WNV often preceded the

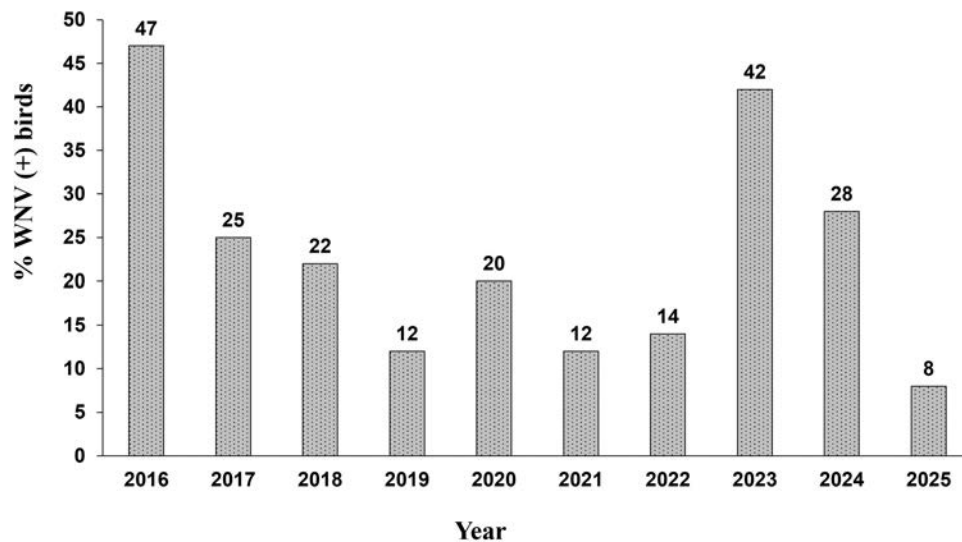


Figure 4.—Percentage of dead birds positive for West Nile virus in California, 2016–2025.

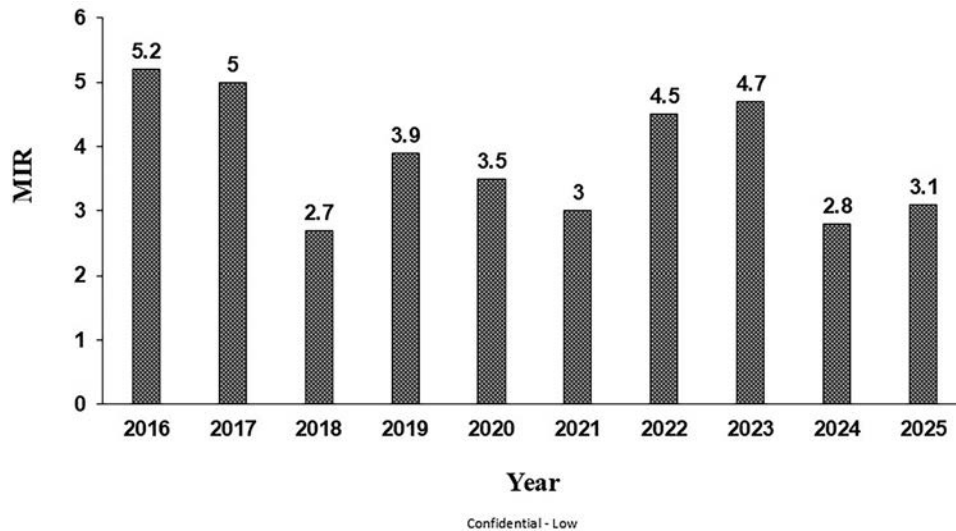


Figure 5.—Minimum infection rate (MIR) in *Culex* females per 1,000 tested for West Nile virus in California, July–September, 2016–2025.

detection of human cases, supporting the value of environmental surveillance to direct mosquito control efforts and decrease the risk arboviral disease transmission in California.

Acknowledgements

The authors gratefully acknowledge the cooperation and assistance of local mosquito and vector control agencies in the collection and submission of samples for testing and their financial support to the testing laboratories and the WNV & Dead Bird Call Center; the local public health and commercial laboratories which tested clinical samples; the many physicians and veterinarians who submitted specimens from suspect cases of arboviral disease, and the valuable contributions of the staff of MVCAC, DART (especially

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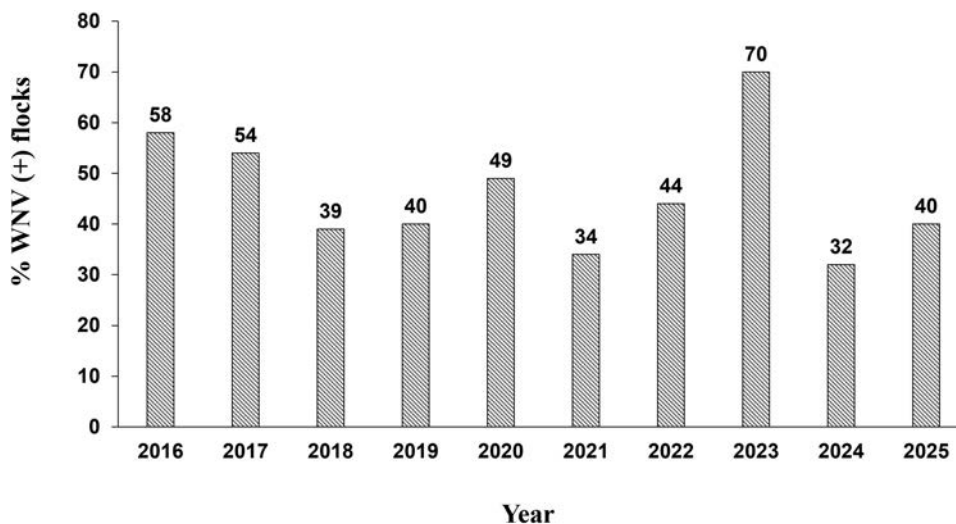


Figure 6.—Percentage of sentinel chicken flocks in California with one or more birds positive for antibodies to West Nile virus, 2016–2025.

Allyx Nicolici), and VBDS (especially Ervic Aquino, Erin Belden, Margaret Kerrigan, Mario Orantes, Mary Joyce Pakingan, and Andrea Salinas).

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2024 Dengue surge in human cases

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Introduction

The initial detection of invasive *Aedes* spp. within the Greater Los Angeles County Vector Control District (GLACVCD) was recorded in 2011 with the identification of *Aedes albopictus* and in 2014 with *Aedes aegypti* and *Aedes notoscriptus*. These findings significantly expanded the known *Aedes* species distributions, emphasizing the need for comprehensive, continuous surveillance and leading to the formation of the GLACVCD *Aedes* Program in 2012. The *Aedes* Program consists of trap-based surveillance, area source inspections, and evidence-based treatments with the initial aim of preventing the spread of invasive *Aedes* species. As invasive *Aedes*, particularly *Aedes aegypti*, took hold and spread throughout the District's service area, the *Aedes* Program was restructured several times to respond to the resident's growing needs. Today, the program continues to play a critical role in mitigating the risk of travel – associated and locally transmitted mosquito-borne diseases within the District service area. In 2024, GLACVCD accounted for one quarter of travel-associated human cases of dengue in California including three locally acquired cases. The *Aedes* program had to overcome new challenges due to the dengue case surge, prompting the allocation of additional resources to ensure an effective and timely response. The current presentation outlines the operational and control response to the unprecedented increase of dengue human cases reported within the GLACVCD jurisdiction in 2024.

Methods

When a travel – associated dengue case is identified within district boundaries, GLACVCD initiates an operational response centered on door-to-door (D2D) inspections. The Operations Department, in conjunction with the Scientific Technical Services (Sci-Tech) Department, determines whether a small scale or a full scale D2D response is appropriate and communicates this plan and the planned date of initial D2D inspections to all pertinent staff.

A small scale D2D response involves inspecting 7-8 properties surrounding the index property, as well as any nearby conducive properties. This response can be effectively executed in 1 to 2 hours by one team of two technicians.

A full scale D2D response involves the inspection of all properties within a 150-meter radius from the index property. The number of properties vary depending on parcel size, ranging around 60-80 or more properties. To

efficiently conduct a response of this scale, four teams of two technicians are needed as well as two logistics technicians. This response typically takes about 2-4 hours to complete. For this type of D2D response, a return date is scheduled one week from initial inspection date and indicated on a door hanger for those residents that were unavailable during the initial inspection. Follow-up inspections are conducted by a single team of two people.

A morning briefing is conducted by the Operations Supervisor to review protocols, including the compliance of HIPPA regulations, coordinating teams and map coverage, as well as neighborhood factors such as language barriers and relevant safety precautions.

The roles of technician teams and logistics technicians during a full scale D2D response are critical to effectively respond to a travel-associated case. Technician teams are responsible for initiating contact with residents followed by the inspection of the property (front and backyard). Logistics roles are assigned to a Supervisor, Senior Vector Control Specialist, or an experienced Vector Control Specialist. During a D2D response, logistics are responsible for conducting treatments including larviciding and adulticiding, collecting and modifying sources, coordinating breaks for staff, providing refreshments, and de-escalation support between technician teams and resident. The presence of logistics technicians allows team technicians to focus on conducting inspections and gaining access to properties in a timely manner.

Results and Discussion

The number of cases reported per year by the Los Angeles County Department of Public Health vary. In 2019, GLACVCD reported just under 40 cases; however, this number declined significantly in 2020 and 2021 due to the COVID-19 pandemic and travel restrictions. In 2024, GLACVCD recorded a record number of human cases and conducted a total of 121 investigations. The months with the greatest number of reported cases were October (35 cases) and November (22 cases). A total of 58 small scale D2D responses and 63 full scale D2D responses were conducted. To put this into perspective, 57% of all cases in Los Angeles County and 20% of the total cases in California were reported from residents within our district (California Department of Public Health, 2026).

Some of the challenges we faced during the 2024 D2D responses included terrain, weather, frequency of cases,

conducive properties, and interactions with uncooperative residents. Several cases occurred in cities with hillsides, and most took place during hot summer/early fall months which can cause physical and mental strain on the technicians. The frequency of incoming cases was greater than usual, making it challenging to conduct 121 investigations in a single year while managing service requests from residents and maintaining the routes. Inspecting homes is usually not time-consuming; however, when it comes to inspecting conducive properties, it is very time-consuming and requires follow-up appointments. Lastly, properties with uncooperative residents can be challenging when entry is

denied. The *Aedes* Program lead in conducting these inspections; staff from the Operations Department and the Sci-Tech Department assisted as well. These challenges were successfully addressed through strong leadership from supervisors, staff rotation, and mutual support.

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Detection of Cache Valley virus in the Northern Central Valley

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Introduction

Cache Valley virus (CVV) is an endemic mosquito-borne *Orthobunyavirus* that has been found in many areas of the U.S., southern Canada, and Mexico since it was first described in 1956 from *Culiseta inornata* in Cache Valley, Utah (Holden and Hess 1959). The virus is neuroinvasive and can cause fetal abnormalities and stillbirths in sheep and goats following infection during pregnancy in immunologically naïve ewes and does (Chung et al. 1990a, 1990b). In humans, the virus also can cause disease, and a small number of human cases have been documented, including 3 fatalities (Hughes et al. 2023).

In the northern Central Valley of California, occasional abortions and fetal abnormalities caused by CVV infection in pregnant goats or sheep have occurred, with sporadic, focal increases in some years. One such episodic outbreak occurred in western Yolo County during the spring of 2024 when abortions in goats and sheep were reported from multiple farms. Infections were suspected to have occurred during early winter (Dec 2023–Jan 2024), which was a time period when mosquito surveillance had not been conducted in the affected area. This prompted an investigation around the livestock premises the following winter that aimed to characterize cool-season patterns in mosquito abundance and possible CVV infection around the same time of year. Similarly, prior cool-season mosquito surveillance had been conducted in Shasta County around livestock premises with histories of CVV infections. The current study characterized the results of these investigations.

Methods

Yolo County field collections. Personnel from Sacramento-Yolo Mosquito and Vector Control District (Sac-Yolo MVCD) set dry-ice-baited CDC light traps with lights removed to collect host-seeking female mosquitoes in the vicinity of farms in western Yolo County from September 2024 through February 2025. All mosquitoes collected were identified by species and sex based on morphology, and

females were sorted into pools of up to 50 individuals for CVV testing by RT-qPCR.

Shasta County field collections. Shasta Mosquito and Vector Control District (Shasta MVCD) collected resting mosquitoes by aspiration from various sites near farms with known CVV infections in livestock, and host-seeking mosquitoes by dry-ice baited traps during the off-season from October 2014 to April 2015. All mosquitoes collected were identified by species and sex based on morphology, and females were sorted into pools of up to 50 individuals for CVV testing by plaque assay and RT-qPCR.

Laboratory testing. All mosquito pools were homogenized, and Shasta County mosquito pools initially were tested by plaque assay on Vero cells. Prior to RT-qPCR, mosquitoes were homogenized and processed to extract RNA. A duplex RT-qPCR assay was performed using published methods (Wang et al. 2009). The assay detects CVV and California serogroup viruses such as California encephalitis virus and Jamestown Canyon virus.

Results

Yolo County. Mosquito species collected near the affected farms in western Yolo County included, in descending order of relative abundance: *Culex tarsalis*, *Anopheles freeborni*, *Culiseta inornata*, *Culex pipiens*, *Anopheles franciscanus*, *Aedes melanimon*, and *Culiseta incidens*. *Culex tarsalis* and *An. freeborni* were collected in greater numbers during early fall, and *Cs. inornata* became more abundant in late Oct and Nov. A total of 286 mosquitoes were tested by duplex RT-qPCR for CVV and California serogroup viruses, and all were negative from Yolo County.

Shasta County. Collections from Shasta County included *Ae. melanimon*, *An. franciscanus*, *An. freeborni*, *An. punctipennis*, *Cs. incidens*, *Cs. inornata*, *Cs. particeps*, *Cx. pipiens*, *Cx. stigmatosoma*, and *Cx. tarsalis*. In the fall, *Cs. inornata* was the most abundant species in CO₂-baited traps, whereas *Cx. tarsalis*, *Cx. pipiens*, and *An. freeborni* were the most common species found in resting collections. The diversity of mosquitoes collected from CO₂-baited traps increased during winter (Jan–Apr) and included *Culiseta* and *Anopheles* species that are suspected vectors of CVV. All

mosquitoes collected from Shasta County during 2014-2015 (n=1,629) were negative by the initial plaque assays on Vero cells. However, the mosquitoes collected during 2015 (n=225) were retested using the duplex RT-qPCR, and a single pool of *An. franciscanus* tested positive for CVV.

Discussion

Fall and winter collections of mosquitoes near livestock premises with histories of CVV infections in Yolo and Shasta counties yielded suspected vectors of CVV and found a single pool of *An. franciscanus* from Shasta County positive for CVV by RT-qPCR. All mosquitoes from Yolo County tested negative for CVV and California serogroup viruses by RT-qPCR. However, livestock infections were recognized only after congenital malformations were found in the offspring of infected goats and sheep, which probably was well after the time period of maternal infection. Mosquito surveillance is not commonly conducted near these affected premises, so the investigations reported here were conducted after the known livestock infections and therefore may not fully represent conditions during periods

of peak CVV infection risk. This study is the first report of CVV detection in *An. franciscanus*.

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Spatio-temporal trend of WNV vector mosquitoes in the West Valley Region of San Bernardino County, Southern California

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Introduction

West Nile virus (WNV) remains one of the most important mosquito-borne diseases in California, with transmission dynamics strongly influenced by environmental conditions, mosquito ecology, and urban water management practices. Southern California experiences highly heterogeneous WNV activity, where complex irrigation systems, stormwater retention structures, and urban habitats can sustain persistent populations of vector mosquitoes. Effective surveillance is essential for identifying mosquito hotspots, monitoring seasonal population trends, and guiding targeted integrated vector management (IVM) interventions. Our study evaluated recent spatio-temporal trends of major WNV vector mosquitoes and WNV activity within the service area of the West Valley Mosquito and Vector Control District (WVMVCD) in San Bernardino County, Southern California.

Methods

Routine mosquito surveillance was conducted across the WVMVCD service area using EVS traps to collect host-seeking mosquitoes, gravid traps to capture gravid female mosquitoes, and BG-Sentinel traps for invasive *Aedes* surveillance. Traps were deployed at historical WNV hotspots, service request locations, exploratory trapping sites, and volunteer surveillance locations. Mosquito abundance data collected during 2025 were compared with historical trends from 2020–2024. The study focused primarily on three major WNV vector species: *Culex quinquefasciatus*, *Culex tarsalis*, and *Culex stigmatosoma*. Mosquito abundance was summarized as mosquitoes per trap-night, and WNV-positive mosquito pool activity was evaluated annually alongside the duration of the transmission season. Integrated vector management strategies included routine larval control, public outreach, door-to-door inspections, source reduction, targeted investigations, and pre-season deployment of In2Care Mosquito Trap stations.

Results

Mosquito surveillance data demonstrated a substantial decline in WNV vector populations in 2025 compared with historical averages. *Culex quinquefasciatus* populations declined by approximately 54% relative to 2020–2024 averages, with historical seasonal peaks becoming markedly ‘flattened’ in 2025. The population trend also indicated a possible shift toward a bimodal seasonal pattern associated with late-season rainfall events. *Culex tarsalis* populations remained below 10 mosquitoes per trap-night throughout the surveillance season despite temporary increases linked to environmental water retention and September rainfall. *Culex stigmatosoma* activity remained consistently low throughout the year. Concurrently, WNV activity showed a substantial downward trend. Only three WNV-positive mosquito pools were detected in 2025 compared with 66 positive pools in 2024, 107 pools in 2023, and 96 pools in 2022. Approximately 93% of all WNV-positive pools across surveillance years were associated with *Cx. quinquefasciatus*. In addition, the duration of the WNV transmission season shortened in recent years, with fewer months of active viral detection observed in 2025.

Conclusion

The findings demonstrated a marked decline in both WNV vector mosquito abundance and WNV activity within the WVMVCD service area in recent years. Intensified pre-season integrated vector management strategies, including proactive surveillance, larval habitat management, targeted control interventions, and community outreach, likely contributed to reducing mosquito populations and shortening the WNV transmission period. These results highlight the importance of sustained surveillance and early-season intervention strategies for minimizing WNV risk in urban Southern California environments. Continued adaptive mosquito control efforts remain essential, particularly in response to environmental conditions such as rainfall and water retention that may promote late-season vector amplification.

Optimizing the West Nile, St. Louis and Western equine encephalitis viruses (WSW) RT-qPCR for recent viral sequences and internal positive controls

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Mutations in recent circulating strains of St. Louis encephalitis virus (SLEV) and western equine encephalomyelitis virus (WEEV) prompted us to evaluate the PCR primers used in the triplex “WSW” RT-qPCR assay widely used to detect these arboviruses and West Nile virus in California. Sequence alignments using publicly available sequences from Genbank as well as viral RNA sequences obtained directly from positive mosquito pools in California were constructed for WNV, SLEV and WEEV. These sequence alignments were used to identify recent mutations in the areas of the genome where RT-qPCR primers and probes bind.

For SLEV, new primers and a new probe were designed and constructed to reflect mutations in recently circulating SLEV strains in California. These new primers and probe were tested side by side against the old primers and probe via RT-qPCR. Amplification in wells containing the new primers and probe showed slightly lower C_T values than wells containing the old primers and probe when testing SLEV-positive mosquito pools from 2017. This is indicative of the new primers and probe having a slightly better binding efficiency than the old primers, which were based on a 1975 strain of SLEV and which had one mismatch each compared to modern SLEV strains.

New SLEV primers and probe

SLE1F-2025 – 5’CTGGCTGTTGGAGGGATTCT

SLE1R-2025 – 5’CAGGTCAATTGCACATCCGC

SLE1Probe-2025 – 5’Cy5-TCTGGCGACCAGCGTGCA AGCTG

Although WEEV hasn’t been detected in California since 2007, it has been detected in South America recently, including Brazil in 2023 and Uruguay in 2023 and 2024. A sequence alignment of recent South American strains of WEEV and historic California strains of WEEV showed that one mismatch was found in the region where the forward primer binds, and another mismatch was found in the region where the reverse primer binds. If WEEV returns to California, it is unknown if the reemergent strain will be more like recent South America strains or more similar to historic California strains, so it is important to have primers and probes that will detect both. A degenerate forward primer was designed to create a mixture of primers that would exactly match both the historic California strain, as

well as the recent Uruguay strain. A degenerate reverse primer was also designed with the same goal. To test these new primers, two ultramer DNA strands were created to act as PCR templates - one strand based on the historic California strain, and a second based on the Uruguay 2024 strain. These ultramers were diluted 10-fold to make a standard curve. RT-qPCR for these WEEV primers/probe sets yielded almost identical C_T s against the same ultramer template. This showed that the new and old primer/probe sets had very similar binding efficiencies. However, the new (degenerate) primer/probe set exhibited a reduction in sensitivity, as it did not amplify the historic California ultramer at the lowest concentration, while the old primers and probes did. Similarly, the new primers showed a greater loss in sensitivity for the Uruguay 2024 ultramer and were not able to amplify the two lowest concentrations. Therefore, the new primers did not offer an advantage when detecting either ultramer, supporting continued use of the existing WEEV primers (“WEEV-2”, Brault et al. 2015) in the “WSW” primers and probe set.

For WNV, some mutations were found in regions where our primers and probes bind, but there is no evidence that these mutations have persisted across the years and strains of WNV that have been sequenced. Therefore, no new primers were considered necessary for WNV. It was necessary to compare the results from both primer sets, to ensure that new primers for WEEV or SLEV are not binding to WNV primers. No evidence of any interference was observed.

Additionally, two internal positive controls (IPC) were evaluated for possible addition to the assay to detect any PCR inhibitors that could be present naturally in mosquito pools, or introduced into the samples during the RNA extraction process. These primers and probes targeted two endogenous *Culex* genes, RiboP and SCBP as the IPCs. These primers and probes have been previously published (Miller et al. 2025). Primers and probes for these endogenous controls yielded very strong C_T values; RiboP yielded a C_T range of 17.4-25.5 and SCBP had a range of 15.1-20.9. This meant that the *Culex* mosquito pools naturally had a very high gene expression of these targets, which could deplete dNTPs before the viral targets could start to amplify. To minimize this depletion of dNTPs, an effort was made to reduce the concentration of primers and

probes for these endogenous controls. But there were still samples where the signal for the weakly positive viral target was lost due to competition from a strong signal from the endogenous mosquito gene. Both IPCs tested showed that the endogenous mosquito genes were able to be detected and amplified in all *Culex* species evaluated: *Cx. tarsalis*, *Cx. pipiens* complex, *Cx. thriambus*, and *Cx. erythrothorax*. Over 100 mosquito pool samples were tested using this 5-plex RT-qPCR assay, including both IPCs. At least one of the two IPCs was detected in every sample tested, thus demonstrating that PCR inhibition does not seem to be a problem, either from PCR inhibitors naturally present in our mosquito pool samples, or from known inhibitors that are part of the RNA extraction process, such as ethanol or isopropanol. Because PCR inhibition does not seem to be a

problem and the use of primers and probes for these IPCs was shown to have a negative effect on the sensitivity for detecting viral targets, IPCs are not recommended for inclusion in future assays for routine surveillance.

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Implementation of a Larval Resistance Surveillance Program: Evaluating container effects on methoprene bioavailability

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Mosquito control programs depend on larvicides such as methoprene to reduce vector populations and prevent disease transmission. Resistance surveillance is essential for maintaining the effectiveness of these interventions. Bench top cup assays are used to determine lethal doses. These assays are typically performed in Styrofoam cups to reduce the binding of methoprene to the cup material. However, due to restrictions on Styrofoam use in California, alternative cup materials including high-density polyethylene (HDPE), polyethylene (PE), polypropylene (PP), and paper were assessed. Results indicated significant variation in larval mortality and emergence inhibition across cup materials (Fig 1). Polyethylene exhibited the lowest binding affinity and highest bioavailability, whereas HDPE and paper required higher concentrations to achieve similar effects. These findings indicated that container material composition can significantly influence insecticide efficacy measurements and supported the adoption of polyethylene cups for standardized resistance testing.

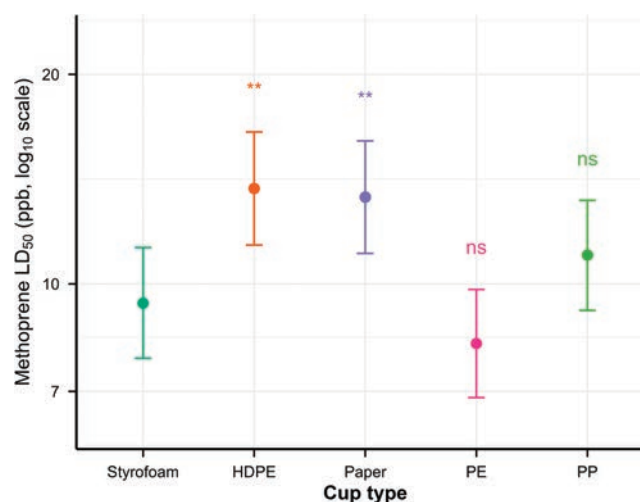


Figure 1.—Estimated LD₅₀ (ppb) by cup type from a quasibinomial probit model. Points show model-based estimates with 95% confidence intervals. Asterisks indicate Dunnett comparisons versus the Styrofoam reference (*P < 0.05, **P < 0.01, ***P < 0.001; ns = not significant). Values are plotted on a log₁₀ scale.

Reinvigorating essential oils for enhanced mosquito repellency using nootkatone synergy

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Abstract

Spatial repellents are increasingly recognized as a complementary tool for mosquito control, yet their evaluation remains largely limited to short-term behavioral endpoints. Here, we examine both immediate and carryover effects of botanical spatial repellents on *Culex quinquefasciatus*, a globally distributed urban vector. Using a modified excito-repellency assay, we quantified behavioral responses to nootkatone, eugenol, and geranium, tested individually and in combination, and then assessed post-exposure blood-feeding success and reproductive output. Essential oil treatments (eugenol and geranium) elicited strong behavioral responses, marked by early increases in flight activity and progressive increases in escape behavior, whereas the sesquiterpene nootkatone alone produced comparatively limited effects. Coformulation with nootkatone enhanced knockdown and amplified behavioral disruption without increasing mortality. Prior exposure consistently reduced blood-feeding success and substantially decreased reproductive output, with the strongest effects observed under coformulated treatments. Reproductive allocation declined across oviposition intervals in all treatments, with the strongest suppression occurring early after exposure and treatment-specific differences becoming less pronounced at later time points. These findings demonstrated that spatial repellents induce temporally structured responses that extend beyond immediate avoidance, linking short-term behavioral disruption to longer-term reductions in feeding and reproduction. Coformulation of botanical compounds enhances these effects through complementary modes of action, highlighting the importance of multi-component strategies. More broadly, this work supports a shift toward evaluating spatial repellents as dynamic, multi-stage interventions capable of influencing mosquito population dynamics through sublethal effects.

Introduction

Urban mosquito control continues to face persistent challenges due to ecological adaptability, behavioral plasticity, and widespread insecticide resistance among key vector species. *Culex quinquefasciatus*, a globally distributed vector of West Nile virus, St. Louis encephalitis, and lymphatic filariasis, exemplifies these challenges (LaDeau et al. 2015; Bhattacharya et al. 2016). This species thrives in polluted, human-modified environments such as storm drains and septic systems, where larval habitats are difficult to locate and eliminate. Its opportunistic feeding behavior and ability to bridge avian and human hosts further complicate control efforts. Conventional control strategies rely heavily on insecticides and larval source management, but these approaches are increasingly constrained by resistance and operational limitations (Bhattacharya et al. 2016; Liu 2015). In this context, spatial repellents have emerged as a promising complementary strategy. Unlike contact insecticides, spatial repellents act at a distance, disrupting host-seeking behavior and preventing mosquito–host contact before biting occurs (Achee et al. 2012; Grieco et al. 2007). However, most evaluations of spatial repellents focus exclusively on immediate avoidance, with limited attention to downstream

effects on mosquito performance and population dynamics (Ogoma et al. 2012; Afify and Potter 2020).

Plant-derived essential oils such as eugenol and geranium oil exhibit repellent activity but are limited by high volatility and short-lived efficacy (Hillary et al. 2024; Isman 2020; Lalthazuali & Mathew 2017). Nootkatone, a natural sesquiterpene with repellent and insecticidal properties, offers a complementary profile, including multimodal action on mosquito sensory and neural pathways (Clarkson et al. 2021; Norris & Coats 2017). Coformulating nootkatone with essential oils may therefore enhance both the intensity and persistence of repellency and broaden the range of sensory disruption (Norris and Coats 2017; Seiber et al. 2014). The current study was designed to address two key gaps: (i) whether coformulated botanical repellents enhance spatial repellency relative to individual compounds, and (ii) whether short-term exposure to these volatiles induces carryover effects on blood feeding and reproduction.

Methods

Adult female *Cx. quinquefasciatus* from a colony derived from Southern California storm drain populations were used

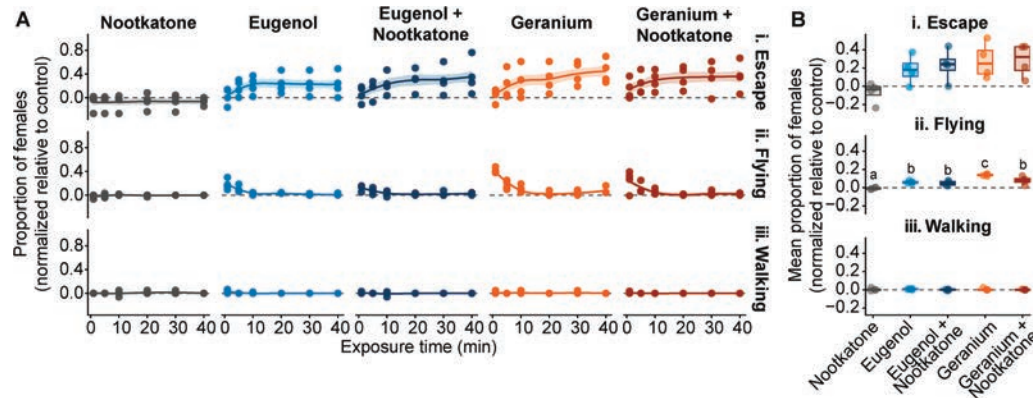


Figure 1.—Behavioral responses to spatial repellents. (A) Temporal dynamics of escape, flight, and walking behavior over a 40-min exposure period, expressed as proportions normalized to control. Points represent replicate observations; lines indicate model fits. (B) Time-collapsed mean responses for (i) escape, (ii) flying, and (iii) walking across treatments. Points represent replicate means, and boxplots show the median and interquartile range. Different letters indicate significant differences among treatments ($p < 0.05$).

to ensure ecological relevance to urban environments. Repellent responses were quantified using a modified excito-repellency assay consisting of a release chamber connected to an escape chamber, allowing simultaneous measurement of spatial redistribution, escape behavior, and activity. Treatments were structured to evaluate both the independent and combined effects of compounds. These included a mineral oil control, single-compound treatments (nootkatone, eugenol, geranium), and coformulated treatments (eugenol + nootkatone, geranium + nootkatone). Nootkatone was incorporated at a standardized concentration of 5% (w/v) across all relevant treatments. For each treatment, four replicate trials were conducted ($n = 4$ replicates per treatment), with approximately 30 adult female mosquitoes used per replicate trial.

Behavioral responses were quantified over a 40-minute exposure period, including escape probability, flight activity, walking activity, and knockdown. Behavioral data were analyzed using generalized linear mixed-effects models with appropriate error distributions (binomial for proportional responses), including treatment, time, and their interaction as fixed effects and replicates as a random effect. Following exposure, mosquitoes were maintained to assess delayed effects on blood-feeding success and reproductive output. Blood-feeding and reproductive assays were conducted with six replicate groups per treatment ($n = 6$), each comprising approximately 25 exposed female mosquitoes. Females were offered bovine blood through a membrane-based glass feeder maintained at 36°C. Blood-feeding success and knockdown were analyzed using generalized linear models with a binomial error structure, whereas reproductive output was analyzed using linear or generalized linear models, depending on distributional assumptions. Reproductive output was quantified as the total number of larvae hatching from oviposited egg rafts across oviposition intervals, providing an integrated measure of realized fecundity.

Results

Behavioral responses to spatial repellents

Behavioral responses were driven primarily by essential oil treatments (Fig. 1). After normalizing relative to controls, escape probability increased under eugenol ($\beta = 0.92 \pm 0.22$, $p < 0.001$), eugenol + nootkatone ($\beta = 1.04 \pm 0.22$, $p < 0.001$), geranium ($\beta = 1.01 \pm 0.23$, $p < 0.001$), and geranium + nootkatone ($\beta = 1.41 \pm 0.21$, $p < 0.001$), whereas nootkatone alone did not differ from the control ($\beta = -0.01 \pm 0.24$, $p = 0.96$; Fig. 1Ai, Bi). Behavioral responses exhibited clear temporal structure. Flight activity was elevated early in the exposure period and declined over time ($\beta = -0.10 \pm 0.05$, $p = 0.031$; Fig. 1Aii, Bii), whereas escape responses increased progressively ($\beta = 0.017 \pm 0.007$, $p = 0.018$). These opposing trajectories indicated a transition from initial behavioral activation to spatial displacement. Walking behavior remained minimal across treatments, with no detectable effects of treatment or time (all $p \geq 0.07$; Fig. 1Aiii, Biii), suggesting that behavioral responses to repellents were dominated by flight-mediated activity and spatial displacement. Knockdown occurred concurrently with behavioral responses and was most pronounced in coformulated treatments, particularly eugenol + nootkatone, reflecting a concurrent process of behavioral activation and impairment.

Knockdown and mortality

Knockdown differed significantly among treatments ($p < 0.05$; Fig. 2A), with the eugenol + nootkatone combination producing the highest knockdown proportions, although responses overlapped with geranium + nootkatone. In contrast, post-assay mortality remained low and did not differ among treatments, with proportions near zero across most replicates (Fig. 2B). Although a modest increase in mortality was observed under geranium relative to control ($\beta = 1.69 \pm 0.80$, $p = 0.036$), this did not result in separation among treatments, indicating

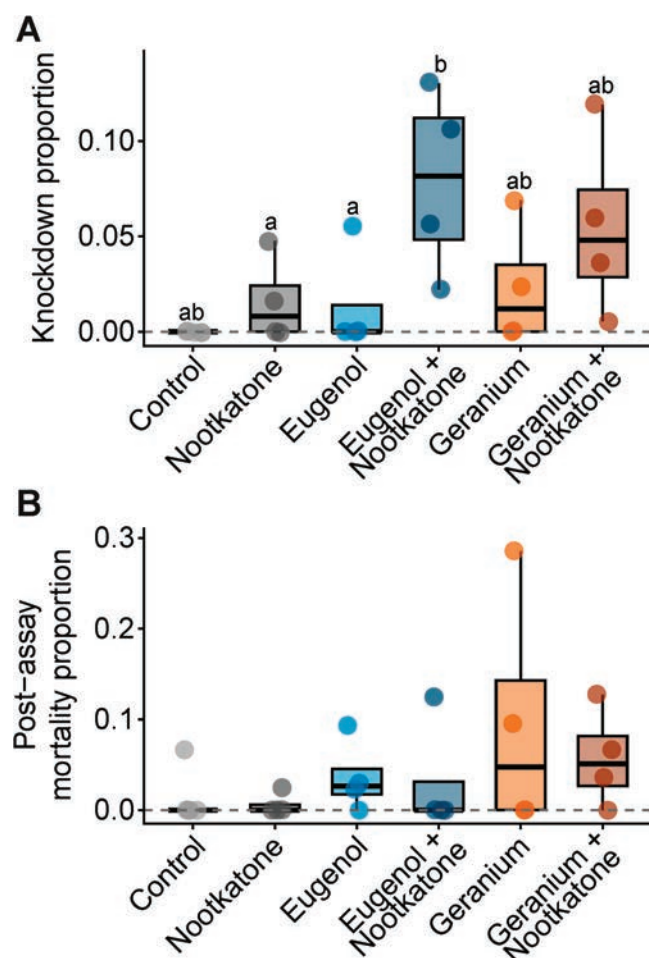


Figure 2.—Knockdown and post-assay mortality across treatments. (A) Knockdown proportion across treatments. Points represent replicate values; boxplots show median and interquartile range. Different letters indicate significant differences among treatments ($p < 0.05$). (B) Post-assay mortality proportion measured 24 h after exposure. Points represent replicate values; boxplots show median and interquartile range. No significant differences among treatments were detected.

that the observed effects were driven primarily by behavioral disruption rather than acute toxicity.

Blood-feeding and reproduction

Exposure to spatial repellents reduced blood-feeding success across treatments (Fig. 3A). Relative to control, blood-feeding probability declined under eugenol ($\beta = -2.90 \pm 0.45$, $p < 0.001$), eugenol + nootkatone ($\beta = -1.01 \pm 0.23$, $p < 0.001$), geranium ($\beta = -0.62 \pm 0.24$, $p = 0.009$), and geranium + nootkatone ($\beta = -0.88 \pm 0.22$, $p < 0.001$) treatments, whereas the reduction under nootkatone alone was weaker and not significant ($\beta = -0.42 \pm 0.24$, $p = 0.078$). Reproductive output, quantified as the total number of larvae produced per blood-fed female from oviposited egg rafts across oviposition intervals, was substantially reduced across all treatments relative to the control (Fig. 3B). Progeny production per blood-fed female declined under nootkatone

($\beta = -0.68 \pm 0.02$, $p < 0.001$), eugenol ($\beta = -1.12 \pm 0.05$, $p < 0.001$), eugenol + nootkatone ($\beta = -1.71 \pm 0.03$, $p < 0.001$), geranium ($\beta = -1.08 \pm 0.02$, $p < 0.001$), and geranium + nootkatone ($\beta = -1.97 \pm 0.03$, $p < 0.001$) treatments, with clear separation among treatments. The strongest reductions occurred under coformulated conditions, particularly geranium + nootkatone. Reproductive allocation declined across oviposition intervals in all treatments, with the greatest differentiation observed early and convergence at later time points, indicating that suppression of reproductive output was strongest immediately following exposure and diminished over time.

Discussion

The results indicated that botanical spatial repellents elicit coupled behavioral and physiological responses that extend beyond immediate avoidance. Behavioral responses were temporally structured, with early activation (flight) followed by progressive displacement (escape), while concurrent knockdown acted as a competing process that reduced the pool of responsive individuals.

Coformulated treatments consistently enhanced these responses, supporting the hypothesis that combining compounds with distinct modes of action and physicochemical properties amplified efficacy. Essential oils primarily modulate olfactory pathways, whereas nootkatone targets neural pathways, including GABAergic signaling and odorant receptor co-receptors. These complementary mechanisms likely produce broader and more persistent disruption of host-seeking behavior. Importantly, these short-term behavioral effects translated into robust downstream consequences for feeding and reproduction, despite minimal effects on survival. This decoupling of survival from performance underscores the importance of sublethal effects in shaping mosquito fitness and population dynamics.

Implications for spatial repellent evaluation and vector control

Current evaluation frameworks for spatial repellents emphasize short-term endpoints, such as percent repellency. The present findings indicated that these measures capture only a subset of repellent effects, particularly for volatile botanical compounds that elicit temporally structured responses and downstream impacts on fitness. By linking spatial exposure to reductions in blood feeding and reproductive output, this study supports a broader framework in which spatial repellents are evaluated not only for immediate avoidance but also for their capacity to induce carryover effects that influence population growth. From an applied perspective, coformulated botanical repellents offer a promising strategy for improving efficacy in complex urban environments. By combining compounds with complementary properties, it may be possible to enhance both the spatial extent and duration of protection while reducing mosquito performance.

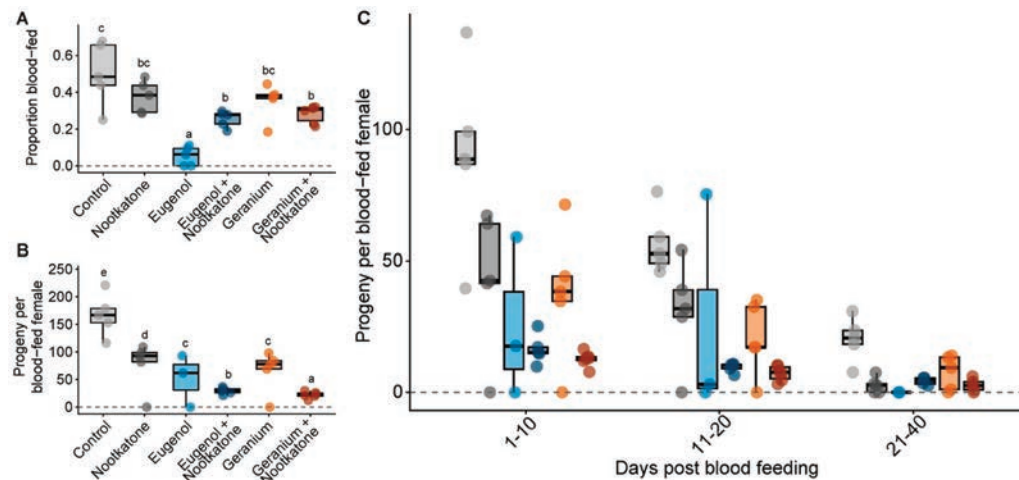


Figure 3.—Effects of spatial repellents on blood feeding and reproductive performance. (A) Proportion of females that blood-fed across treatments. (B) Progeny production per blood-fed female, quantified as the number of larvae hatched from oviposited egg rafts. (C) Temporal dynamics of reproductive output across oviposition intervals (1–10, 11–20, and 21–40 days post-blood feeding). Points represent replicate values; boxplots show median and interquartile range. Different letters indicate significant differences among treatments ($p < 0.05$).

Conclusions

Short-term exposure to botanical spatial repellents elicits coordinated responses that range from immediate behavioral disruption to longer-term effects on feeding and reproduction. Coformulation with nootkatone enhances these effects without increasing mortality, indicating that efficacy stems from behavioral and physiological disruption rather than toxicity. These findings support a shift toward viewing spatial repellency as a dynamic, multi-stage process and highlight the potential for sublethal effects to contribute to mosquito population suppression. Integrating behavioral, physiological, and life-history endpoints into evaluation frameworks will be critical to advancing the development of next-generation spatial repellents for urban vector control.

Acknowledgements

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Population suppression, not exclusion, underlies spatial repellent effects in urban storm drain mosquito habitats

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Abstract

Urban mosquito control increasingly emphasizes habitat-targeted interventions to reduce vector populations within key refugia rather than relying solely on area-wide suppression. Underground storm drain systems (USDS) represent important urban mosquito habitats because they provide stable microclimates and protected resting and oviposition sites, particularly for *Culex quinquefasciatus*. We evaluated the effects of botanical spatial repellents applied within USDS chambers during spring and fall 2025 field trials in Palm Desert, California. Spring treatments compared slow-release drip dispensers with combined drip+spray applications, whereas fall treatments focused on spray-based applications using eugenol or peppermint oil. Mosquito abundance and occupancy were monitored weekly across pre-treatment, treatment, and post-treatment phases using CDC ultraviolet light traps. Across both trials, mosquito communities were strongly dominated by *Cx. quinquefasciatus*, and treatment effects were primarily associated with reductions in its abundance. Spatial repellent treatments reduced mosquito abundance within storm drains while occupancy often remained relatively high, indicating that treatments primarily reduced mosquito abundance within occupied habitats rather than eliminating site use entirely. Suppression patterns differed between seasons, with spring populations exhibiting partial rebound following treatment cessation whereas fall treatments showed more persistent reductions. Reductions within storm drains were also associated with small declines in nearby above-ground mosquito activity. Together, these findings provided field-based evidence that habitat-targeted spatial repellency can reduce mosquito abundance in underground storm drain systems and underscored the importance of distinguishing between occupancy and abundance when evaluating vector control interventions.

Introduction

Urban mosquito control has increasingly shifted toward habitat-targeted interventions rather than uniform suppression across entire landscapes (Jackson et al. 2013; Johnson et al. 2018; Vanlerberghe et al. 2017). This shift reflects both practical constraints and growing recognition that mosquito populations are structured around a limited number of key refuges that disproportionately support resting, oviposition, and development (Becker et al. 2010; Westby et al. 2021; Gu et al. 2008). In many urban environments, underground storm drain systems serve as such refuges, providing stable microclimates, persistent organic inputs, and protection from surface disturbance (Dey et al. 2025; Irwin and Harbison 2024; García and Santamarta 2022). These habitats can sustain mosquito populations even when conditions above ground are unfavorable, yet their role in shaping population responses to control remains incompletely understood (Su et al. 2003; Wang et al. 2021). Although underground storm drain systems in southern California are frequently associated with *Culex* mosquitoes, particularly *Cx. quinquefasciatus* (Su et al. 2003; Wang et al. 2021), these habitats can support diverse mosquito assemblages whose responses to habitat-targeted interventions may differ across species and seasons.

A central challenge in evaluating habitat-targeted mosquito control lies in distinguishing between changes in site occupancy and changes in local abundance (Low et al. 2016; K. G. Evans et al. 2022). Most field evaluations rely on measures such as trap positivity, presence or absence, or short-term changes in abundance (Achee et al. 2012; Killeen et al. 2003). Although operationally convenient, these metrics often conflate distinct ecological processes. Mosquito abundance may decline because individuals abandon treated habitats entirely, or because fewer mosquitoes occupy the same habitats while still remaining locally present (Baitharu et al. 2020; Gu et al. 2006). These mechanisms carry very different implications for population persistence, recovery, and long-term control efficacy, yet they are rarely disentangled in empirical studies.

This distinction is especially consequential in urban systems, where mosquito populations are maintained by a spatially complex network of both visible and cryptic habitats (M. V. Evans et al. 2022). Storm drains, catch basins, subterranean infrastructure, irrigation systems, and other concealed refuges can support mosquito persistence even within the immediate vicinity of treated sites (Su et al. 2003). In such landscapes, apparent suppression at targeted locations may coexist with continued population

maintenance nearby, facilitated by movement among interconnected habitats (Su et al. 2003; Marini et al. 2020; Wang et al. 2021). Without explicitly separating changes in site occupancy from changes in local abundance, interventions may be incorrectly judged effective or ineffective based on localized observations alone, obscuring how mosquito populations are reorganized across the broader urban habitat mosaic (Biswal 2023; M. V. Evans et al. 2022).

Density-dependent processes further shape these outcomes. Competition for resting space, oviposition sites, and larval resources strongly influence mosquito survival, development, and fecundity across life stages (Juliano 2009; Alto et al. 2008). As a result, reductions in abundance within key habitats can yield meaningful ecological and epidemiological benefits even when mosquitoes continue to use those habitats or redistribute among nearby alternative habitats, where competition for limited resources may remain high. Under both scenarios, suppression of mosquito abundance can translate into reduced biting pressure and lower pathogen transmission potential (Killeen et al. 2003; Achee et al. 2012). At the same time, short-term mosquito suppression may alter resource availability in ways that facilitate population rebound once treatment pressure is relaxed. Ecological theory predicts that reductions in intraspecific competition can increase per capita resource availability and promote compensatory population growth following disturbance (Tilman 1982; Murdoch et al. 2003), a mechanism documented across insect pest and vector systems (Southwood et al. 1974; Hastings 2004).

Community structure adds another layer of complexity. Urban mosquito assemblages are often highly uneven, with one or a few dominant species accounting for most abundance and public health risk (Becker et al. 2010). In such systems, aggregate responses to interventions are driven primarily by dominant taxa, whereas rarer species contribute disproportionately to changes in community composition rather than to total abundance (Becker et al. 2010; Qualls et al. 2023). Evaluations that focus on shifts in species composition alone may therefore provide a misleading picture of control efficacy if changes in relative abundance are not considered (Qualls et al. 2023; Lajaaiti et al. 2024). Interpreting intervention outcomes thus requires a framework that explicitly integrates species identity with contribution to overall abundance (Hubbell 2011; Chase and Leibold 2009). Seasonality further modulates these dynamics through weather-driven changes in temperature, resource availability, and mosquito population growth rates. (Reiner et al. 2013; Mordecai et al. 2019). Interventions applied at different times of year may produce similar short-term suppression yet differ markedly in mosquito population persistence and recovery. Despite this, relatively few field studies explicitly examine how seasonal context interacts with habitat targeted control to shape abundance suppression, rebound, and spillover into mosquito activity above ground.

Here, we present a field-based evaluation of habitat-targeted mosquito control using spatial repellency-based

interventions applied within underground storm drain systems (USDS). We use the term spatial repellent to describe habitat-applied formulations whose primary effects are behavioral deterrence and abundance suppression rather than contact toxicity. The study was structured around two temporally distinct field trials conducted in spring and fall, designed to examine how these interventions operate under different ecological and operational contexts rather than to provide formal replication across seasons. The spring trial evaluated multiple treatment delivery modalities to identify mechanisms of suppression in storm drain habitats and to assess the relative performance of intervention types. Results from this phase informed a focused fall trial that narrowed attention to spray-based repellent applications, selected for their demonstrated efficacy and their operational practicality within routine vector control programs. Within each seasonal trial, mosquito abundance was quantified in storm drain habitats, and, specifically in the fall trial, above-ground mosquito abundance also was monitored to assess whether mosquito suppression within key refuges extended to broader suppression in the local area. A central feature of our approach was the explicit separation of site occupancy from local abundance, coupled with analyses of conditional abundance given presence (i.e., mosquito abundance within sites where mosquitoes were detected), to determine whether interventions primarily reduced mosquito abundances within storm drain habitats that continued to be used or instead excluded mosquitoes from those habitats, potentially displacing them to other nearby refuges. By integrating species-specific responses, dominance structure, and temporal dynamics within each trial, our study emphasizes an ecological and operational framework for evaluating urban mosquito control that prioritizes mechanism, habitat function, and context dependence over binary presence–absence outcomes.

Methods

Study site and experimental design: Field experiments were conducted in a residential neighborhood in Palm Desert, California, to evaluate the effects of habitat-targeted spatial repellency on mosquito abundance within underground storm drain systems (USDS) (Fig. 1). The study consisted of two seasonal field trials, one conducted in spring and one in fall, using the same chambers to facilitate seasonal comparison while maintaining spatial consistency. The spring trial was conducted over seven weeks from 7 May to 19 June 2025. A subsequent fall trial was conducted in the same neighborhood from 23 September to 5 November 2025 using the same chambers.

Fourteen USDS chambers were selected based on prior mosquito surveillance and spatial distribution to ensure consistent baseline mosquito activity and representative coverage across the study area (Fig. 1). Of these, twelve chambers were assigned to treatment groups and two served as untreated controls throughout both seasonal trials. To minimize confounding from local environmental heterogeneity and potential subterranean interconnectivity,

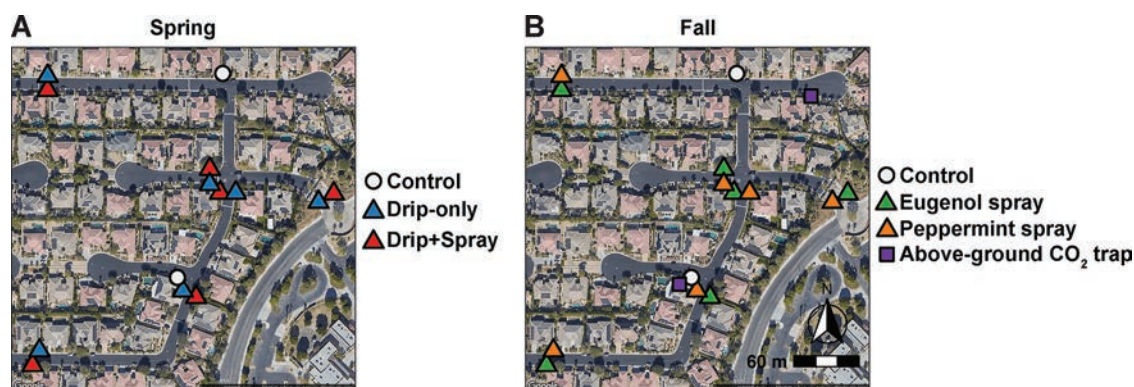


Figure 1.—Spatial layout of underground storm drain system (USDS) chambers used for field evaluation of spatial repellents in Palm Desert, California. Maps show the locations of USDS chambers included in the spring (A) and fall (B) trials. Symbols denote individual chambers and are coded by treatment type: control (gray circles), drip-only (blue triangles), and drip+spray (red triangles) in spring (A); control (gray circles), eugenol spray (green triangles), peppermint spray (orange triangles), and above-ground CO₂ trap (purple squares) in fall (B). Chambers were distributed across residential street segments, and treatments were arranged using a blocked, paired design to limit spatial overlap among treatment types.

the twelve treatment chambers were arranged into six matched pairs. Each pair was located on opposite sides of the same residential street segment and was assumed to represent a shared subterranean cluster. This pairing structure supported a blocked experimental design and enabled within-pair comparisons across treatments. No additional mosquito control operations were conducted within the study area by the local vector control district during either of the seasonal trials.

Spring trial treatments: Within each matched pair, one chamber was randomly assigned to the drip-only treatment and the other to the drip+spray treatment, resulting in six chambers per treatment. In the drip-only treatment, chambers received two gravity-fed, slow-release dispensers loaded with undiluted botanical oils: one containing eugenol and the other peppermint oil. Eugenol and peppermint oils were selected for this study based on prior laboratory evaluations demonstrating their spatial repellency and excito-repellent effects (Chandrasegaran et al., *in prep*). To achieve a continuous release of spatial repellents under field conditions, commercially available 3.5 liter plant irrigation bags (Kityemo; ZWDSB-1-1-5) were repurposed as slow-release dispensers. Each dispenser was fitted with a noncontact flow-adjustment valve (McMaster-Carr; 5192K41) calibrated to deliver approximately 22–26 mL per hour, allowing sustained release of undiluted oil over a 6.5 to 7-day period. Released oil flowed through tubing (McMaster-Carr; $\frac{1}{4}$ " ID and $\frac{3}{8}$ " OD; 5231K161) into a 1.32-gallon polyethylene terephthalate bucket (Mifoci; 15.75"L × 13.39"W × 13.39"H) containing layered cotton pads, which acted as a surface reservoir to promote volatilization while capturing excess oil and preventing direct discharge into the storm drain environment. Dispensers were inspected, serviced, and replenished weekly to maintain consistent release rates throughout the three-week treatment period. In the drip+spray treatment, chambers received the same slow-release dispensers and were supplemented with a weekly spray application of

eugenol. Spray treatments consisted of approximately 0.5 gallons of undiluted eugenol, applied with a backpack sprayer to uniformly coat all interior walls and the ceiling of the chamber. Two chambers received no treatment and served as untreated controls throughout the spring trial.

Fall trial treatments: The same fourteen USDS chambers were used in the fall trial, preserving chamber identity and spatial pairing across seasons. Treatment assignments were retained at the pair level but modified to focus exclusively on spray-based interventions; no drip dispensers were deployed during the fall trial. Chambers that received the drip-only treatment during spring were assigned to receive weekly peppermint spray applications during the fall trial. Chambers that received the drip+spray treatment during spring were assigned to receive weekly eugenol spray applications during fall. Spray treatments followed the same backpack application protocol and volume used in the spring trial. The two control chambers remained untreated throughout the fall experiment. This seasonal reassignment allowed focused evaluation of spray-based spatial repellency under fall conditions while preserving consistency in chamber selection, spatial pairing, and neighborhood context.

Temporal sampling structure: Both the spring and fall trials followed a temporally structured before, during, and after trapping design to quantify baseline conditions, treatment effects, and short-term post-treatment dynamics. During weeks 1 and 2, baseline mosquito activity was recorded in all fourteen chambers. During weeks 3 through 5, repellency treatments were applied weekly in the twelve treatment chambers while monitoring continued across all chambers. During weeks 6 and 7, treatments were discontinued, and monitoring continued to assess residual effects and early recovery (population rebound) following treatment cessation.

Mosquito surveillance within USDS: Mosquito occupancy and abundance within each USDS chamber were monitored weekly using CDC ultraviolet light traps

suspended approximately 30 cm below the manhole cover. Traps were operated overnight for approximately 15 hours and retrieved the following morning (~1600 hrs to 0700 hrs). Mosquitoes were transported on dry ice and processed in the laboratory for species-level identification and enumeration. This approach enabled repeated quantification of mosquito abundance within chambers across all experimental phases while maintaining a consistent sampling effort across treatments and controls. Abundance values represent the combined captures of adult male and female mosquitoes per trap-night.

Above-ground mosquito surveillance during the fall trial: To assess whether treatment-induced changes in mosquito abundance within USDS were reflected in a broader suppression of mosquito host-seeking activity within the residential community, above-ground mosquito abundance was monitored during the fall trial using carbon dioxide-baited traps. Two above-ground trapping locations were randomly selected within the study neighborhood. At each location, a carbon dioxide-baited trap was deployed overnight weekly, concurrent with USDS sampling. Above-ground traps were not deployed during the spring trial, because treatments differed in their delivery methods and intensity of within-chamber exposure, complicating the interpretation of above-ground responses. Following the spring trial, spray-based treatments were selected for focused fall evaluation due to their greater suppression in storm drain systems. Restricting above-ground surveillance to the fall trial allowed above-ground mosquito activity to be interpreted relative to a single standardized intervention modality.

Botanical repellent oils: Spatial repellents consisted of undiluted essential oils of eugenol and peppermint obtained from WholesaleBotanics (Beltsville, Maryland, USA). Eugenol (CAS No. 97-53-0) was steam-distilled from flower buds of *Syzygium aromaticum* and supplied at greater than 99 percent purity. Peppermint essential oil (CAS No. 68917-18-0) was steam-distilled from *Mentha × piperita* and contained high concentrations of monoterpenes, such as menthol and menthone, which exert repellent and irritant effects on mosquitoes and other insects (Pang et al. 2020; Sharma et al. 2023).

Both eugenol and peppermint oil are classified by the U.S. Environmental Protection Agency as minimum-risk pesticide active ingredients under FIFRA Section 25(b). This designation exempts them from federal registration requirements when formulated and applied in accordance with label guidelines, reflecting their low mammalian toxicity and favorable environmental profile. From an operational perspective, this regulatory status is important because it facilitates deployment in urban infrastructure without the permitting, reporting, and compliance burdens associated with conventional insecticides. It also aligns these interventions with integrated vector management programs that prioritize reduced chemical risk, public acceptability of treatment options, and compatibility with routine municipal operations.

All oils were stored in airtight, ultraviolet-protected containers at ambient temperature and used undiluted to maximize volatility and behavioral activity under field conditions.

Statistical analysis: Analyses quantified the effects of habitat-targeted spatial repellency on mosquito abundance, occupancy, and temporal dynamics within underground storm drain systems (USDS). Where available, above-ground mosquito activity was analyzed as a secondary, contextual measure. Spring and fall trials were analyzed separately because treatments and ecological conditions differed between seasons, precluding formal comparisons of treatments across seasons.

Structure and distribution of abundance data: Mosquito counts were highly aggregated, overdispersed, and characterized by a high frequency of zero counts, reflecting episodic use of storm drain habitats and strong temporal variability. For visualization and descriptive summaries, abundance values were $\log_{10}(n+1)$ -transformed to facilitate comparison across taxa spanning multiple orders of magnitude. Inferential analyses were conducted on the original count scale using generalized linear mixed-effects models with negative binomial error distributions, which account for overdispersion directly without requiring transformation of the response variable.

Species-specific abundance patterns: Treatment-associated variation in mosquito abundance within USDS was summarized using species-specific counts across treatments and sampling weeks (Fig. 2). Abundance values represent the total number of adult mosquitoes (males and females combined) collected per trap-night. For each season, abundance was aggregated by species and treatment, and mean values with associated standard errors were calculated to facilitate comparison among treatments. Because counts spanned multiple orders of magnitude across taxa, abundance values shown in Fig. 2 were $\log_{10}(n+1)$ -transformed for visualization only. These descriptive summaries were intended to characterize overall abundance structure across treatments and species and did not explicitly model temporal dynamics. Temporal treatment effects were instead evaluated separately using control-referenced log response ratios (LRR; Fig. 3) and phase-based log-ratio analyses (Fig. 5), both of which quantify relative changes in abundance through logarithmic effect-size metrics rather than transformed abundance values. Because Fig. 2 summarizes abundance averaged across weeks and presents treatments independently, inference regarding treatment efficacy was based primarily on the control-referenced and phase-based analyses described below. For taxa with sparse or highly intermittent detections, interpretation was limited to descriptive summaries, whereas dominant and consistently detected taxa were examined in greater detail.

Community-level summaries and dominance structure: Community composition was summarized using relative abundance (the proportion of total mosquitoes represented by each species) and mean abundance (the

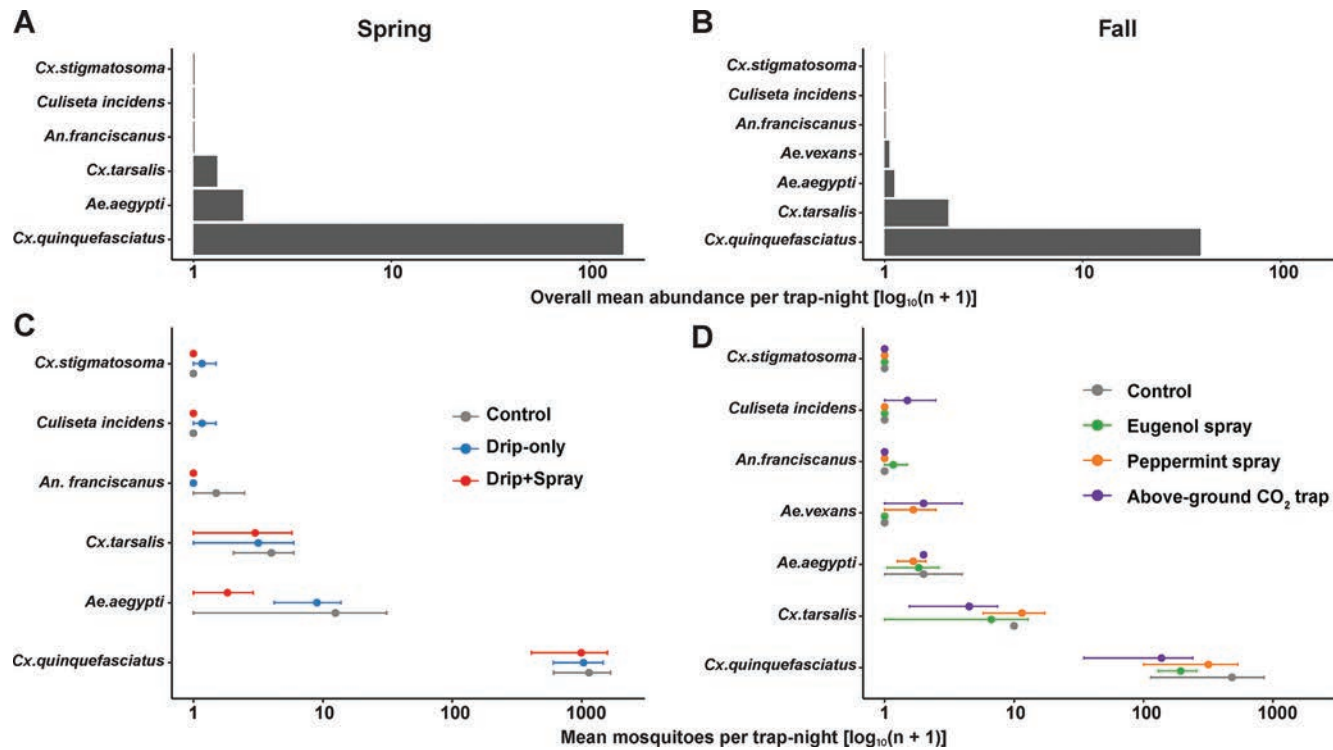


Figure 2.—Season-specific community context and species-specific abundance across treatments. Overall mean abundance of mosquito species pooled across all sampling weeks (weeks 1–7; pre-treatment, treatment, and post-treatment) during the spring (A) and fall (B) trials, expressed on a log₁₀(n+1) scale. (C) Mean abundance by species and treatment during the spring trial, averaged across all sampling weeks (weeks 1–7). Treatments include control (gray), drip-only (blue), and drip+spray (red). (D) Mean abundance by species and treatment during the fall trial, averaged across all sampling weeks (weeks 1–7). Treatments include control (gray), eugenol spray (green), peppermint spray (orange), and above-ground CO₂ trap (purple). Points in panels C and D represent treatment-specific means, and horizontal bars indicate $\pm$ SE. Because values in panels C and D are averaged across the full sampling period, these panels summarize overall abundance differences among treatments and species, rather than temporal dynamics (see Fig. 3 for time-resolved responses). All abundance values are shown on a log₁₀(n+1) scale to facilitate comparison across species spanning multiple orders of magnitude.

average number of mosquitoes collected per trap-night) across treatments and sampling weeks. (Fig. 2).

Temporal dynamics and log response ratios: To characterize the timing, magnitude, and persistence of treatment-induced suppression, weekly log response ratios (LRR) were calculated as descriptive effect-size metrics (Fig. 3; Supp. Fig. 1). Because sampled storm-drains included contemporaneous untreated control units, LRR was calculated relative to the weekly mean abundance in control chambers:

$$LRR(w) = \log \left(\frac{N_{treated}(w) + 1}{\bar{N}_{control}(w) + 1} \right)$$

where $N_{treated}(w)$ is the total mosquito abundance per chamber (summed across all species) in a given treatment during week w , and $\bar{N}_{control}(w)$ is the corresponding weekly mean abundance in control chambers; a pseudocount of 1 was added to accommodate zero counts. Negative LRR values indicate suppression relative to controls, with larger magnitudes corresponding to stronger effects. Because LRR values are expressed on the logarithmic scale, values below zero indicate lower abundance in treated sites relative to

controls. LRR trajectories were used to describe the onset, duration, and recovery of suppression across storm-drain treatments and to characterize temporal deviations from baseline in above-ground mosquito activity. These trajectories were calculated using total mosquito abundance and were used for descriptive purposes, whereas formal inference was based on model-based analyses of abundance and phase-specific metrics. Because the study was designed to evaluate habitat-level mosquito suppression within underground storm drain systems under operational field conditions, aggregate adult mosquito abundance was used as an ecologically relevant measure of total mosquito activity within these habitats. Species-specific temporal patterns were examined separately for the two most abundant species within each trial.

Occupancy analyses: To distinguish changes in site use from changes in local mosquito abundance, mosquito occupancy was analyzed as a binary response indicating presence or absence within each chamber-week (Fig. 4). Occupancy models were fit using generalized linear mixed-effects models with a binomial error distribution and logit link. Treatment and week were included as fixed effects, with chamber identity included as a random intercept. These analyses assessed whether treatments

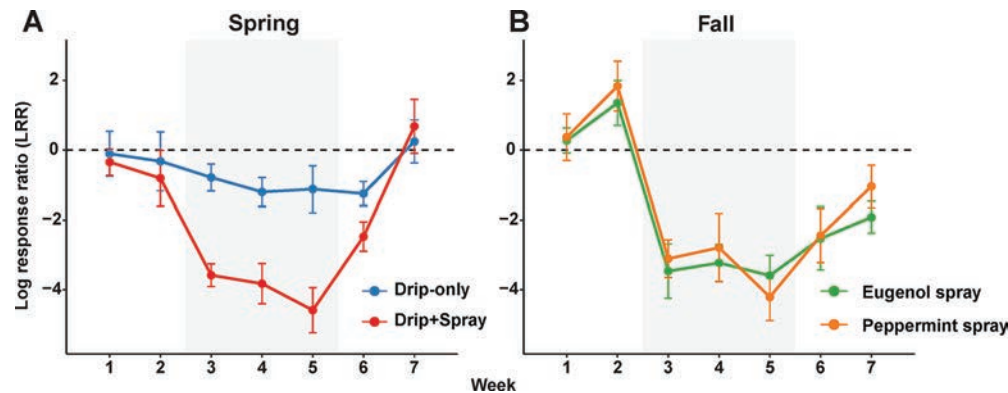


Figure 3.—Temporal dynamics of treatment effects relative to contemporaneous controls. Weekly treatment effects are expressed as log response ratios (LRR), calculated as $\log((\text{abundance in treated sites} + 1)/(\text{mean abundance in control sites for the same week} + 1))$, where negative values indicate lower abundance in treated sites relative to controls, zero indicates no difference, and positive values indicate higher abundance in treated sites. Panels show spring (A) and fall (B) trials across the seven-week sampling period. Points represent weekly mean LRR values for each treatment, and error bars indicate $\pm$ SE. The horizontal dashed line denotes parity with controls, and shaded regions indicate the treatment phase (weeks 3–5). In spring (A), treatments include drip-only (blue) and drip+spray (red) applications. In fall (B), treatments include spray-based eugenol (green) and peppermint (orange) applications.

altered the probability of USDS occupancy independently of effects on abundance when mosquitoes were present.

Conditional abundance given presence: To understand how the treatments reduced mosquito numbers, we examined not only whether mosquitoes were present in storm drains but also how many were present when they occurred. We did this by calculating conditional abundance, defined as the average number of mosquitoes collected per trap-night across storm drains where mosquitoes were detected. This approach distinguishes reductions in site use from reductions in mosquito abundance within occupied storm drains. Conditional abundance was examined by treatment, week, and species, and interpreted alongside occupancy results to determine whether treatments mainly

reduced mosquito abundance within storm drains that remained in use, rather than eliminating storm drain use altogether (Supp. Fig. S2). Analyses focused on the two most abundant species in each trial, because other species appeared infrequently and contributed little to overall mosquito numbers. Because these data were sparse and unevenly distributed across weeks, conditional abundance patterns were interpreted descriptively without formal statistical testing.

Phase-structured analyses: To integrate treatment effects across the experimental timeline, weeks were grouped into three phases: pre-treatment, treatment, and post-treatment. For each USDS, mean abundance was calculated within each phase, and log ratios were computed

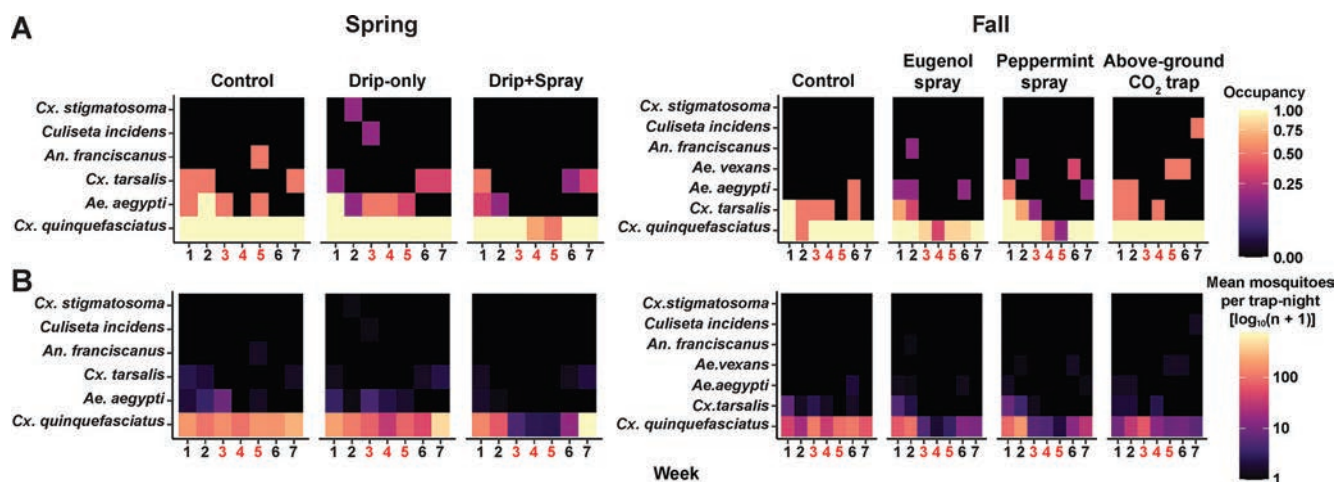


Figure 4.—Species-specific occupancy and abundance across treatments and seasons. Heatmaps summarize species-specific responses across treatments for the spring (left) and fall (right) trials, separating occupancy (A) from abundance (B). Rows correspond to mosquito species, and columns represent sampling weeks (weeks 1–7) within each treatment; treatments are shown in separate panels for each season. Weeks 1–2 represent the pre-treatment period, weeks 3–5 the treatment phase (highlighted in red on the x-axis), and weeks 6–7 the post-treatment period. Occupancy panels (A) show the proportion of sampling units in which a species was detected within each treatment and week (range: 0–1). Abundance panels (B) show mean mosquito counts per sampling unit, expressed on a $\log_{10}(n+1)$ scale.

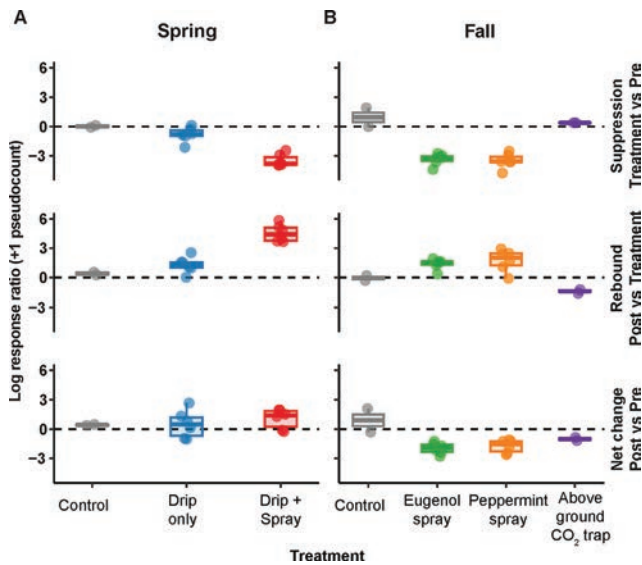


Figure 5.—Phase-structured suppression, rebound, and net change in *Cx. quinquefasciatus*. Phase-structured response metrics summarize changes in *Cx. quinquefasciatus* abundance across experimental phases for the spring (A) and fall (B) trials. Panels show suppression (treatment vs pre-treatment), rebound (post-treatment vs treatment), and net change (post-treatment vs pre-treatment), expressed as log response ratios calculated with a (+1) pseudocount. Points represent individual USDS units, and horizontal bars indicate treatment means $\pm$ SE. The horizontal dashed line denotes no change between phases; values below zero indicate lower abundance relative to the reference phase, and values above zero indicate higher abundance.

to quantify suppression during treatment relative to pre-treatment and recovery following treatment cessation. Phase-specific log ratios were analyzed using linear models with treatment as a fixed effect (Fig. 5). Because each chamber contributed a single value per metric, mixed-effects structures were not applicable. These analyses were restricted to *Cx. quinquefasciatus*, the numerically dominant species across both trials, as this species accounted for the majority of total abundance and therefore drove aggregate treatment responses.

Above-ground mosquito abundance: Where above-ground surveillance data were available, mosquito abundance was analyzed using generalized linear mixed-effects models (negative binomial, log link), with treatment and sampling week as fixed effects and sampling location as a random intercept. Above-ground abundance was quantified using CO₂-baited traps placed at fixed locations within the study neighborhood and is interpreted as an index of host-seeking adult mosquito activity outside underground storm drain systems. Above-ground CO₂-baited traps primarily sampled host-seeking adult mosquito activity, whereas underground UV-light trap collections likely reflected a mixture of resting, emerging, and actively moving adults within storm drain habitats. Treatment was defined based on the repellent applied within each USDS cluster. These analyses were used to assess patterns of above-ground mosquito abundance across treatments in

parallel with suppression observed within storm drain habitats, acknowledging that above-ground captures reflect a composite of adult movement, dispersal, and use of alternative refuges beyond treated sites.

All statistical analyses were conducted in R (version 4.2.2). Generalized linear mixed-effects models were fit using the *glmmTMB* package with a negative binomial distribution and log link. Treatment and sampling week (modeled as a categorical fixed effect) were included as fixed effects, and chamber identity was included as a random intercept to account for repeated sampling. Estimated marginal means and pairwise comparisons among treatments were obtained using the *emmeans* package. Model fit was evaluated using simulation-based residual diagnostics implemented in the *DHARMA* package.

Results

Community structure and seasonal dominance:

Across both spring and fall sampling periods, mosquito communities within USDS chambers, based on combined adult male and female captures, were strongly dominated by *Culex quinquefasciatus*, which accounted for the majority of captures across all treatments and weeks (Fig. 2A–B). In spring, this dominance was pronounced, with *Cx. quinquefasciatus* occurring at substantially higher abundances than all other taxa (mean log-transformed abundance $\approx$ 2.0–2.3, compared with $\leq$ 1.2 for other species). *Aedes aegypti* and *Culex tarsalis* were consistently detected but at much lower abundances, while *Anopheles franciscanus*, *Culiseta incidens*, and *Culex stigmatosoma* were observed only sporadically and at very low numbers (Fig. 2A). In fall, this dominance pattern persisted (Fig. 2B). Mean abundance of *Cx. quinquefasciatus* was lower than in spring, and subordinate taxa such as *Cx. tarsalis* and *Ae. aegypti* contributed slightly more to overall community composition. However, the abundance distribution remained highly skewed, with *Cx. quinquefasciatus* comprising the majority of total counts in both seasons. Accordingly, aggregate patterns closely tracked species-specific patterns for *Cx. quinquefasciatus*, indicating that overall treatment responses were driven primarily by this species.

Species-specific abundance responses to treatment:

Treatment effects differed among species and were most pronounced for *Cx. quinquefasciatus* (Fig. 2C–D). These estimates were based on models fit across all sampling weeks (weeks 1–7), encompassing pre-treatment, treatment, and post-treatment periods, and therefore represent average abundance across the full experimental timeline.

In the spring trial, *Cx. quinquefasciatus* abundance was lower in both intervention treatments relative to untreated controls. Mean abundance declined from control chambers to drip-only treatments and further under drip+spray treatments, corresponding to approximately 2–3-fold lower abundance under drip-only treatments and $\sim$ 8-fold lower abundance under drip+spray treatments relative to controls. The reduction under drip+spray treatment was strongly

supported statistically ($p < 0.0001$), whereas the reduction under drip-only treatment was weaker and marginal ($p \approx 0.05$). Abundance under drip+spray treatment was also lower than under drip-only treatment ($p < 0.0001$), indicating stronger suppression when application methods were combined. In contrast, *Aedes aegypti* showed no consistent differences among treatments, with overlapping estimates and no statistically supported treatment effects. Other taxa were detected at low abundance, and variation among treatments was not statistically supported, reflecting low counts and intermittent detection rather than clear treatment responses (Fig. 2C). These patterns were consistent with species-specific weekly log response ratios (LRR) (Supp. Fig. S1). In spring, *Cx. quinquefasciatus* showed sustained negative LRR values during treatment weeks (weeks 3–5) under the drip+spray treatment, indicating lower abundance relative to paired control chambers during this period. In contrast, LRR values for *Ae. aegypti* were variable across weeks and did not show a sustained directional shift (Supp. Fig. S1A).

In the fall trial, treatment effects on *Cx. quinquefasciatus* were stronger and more consistent (Fig. 2D). Mean abundance declined from control chambers to all intervention treatments, with spray-based treatments producing the largest reductions, corresponding to approximately 9–13 fold lower abundance relative to controls. The overall treatment effect was statistically supported ($\chi^2 = 27.6$, $p < 0.001$), and pairwise contrasts indicated strong reductions under both spray treatments ($p < 0.0001$). For *Cx. tarsalis*, reductions in abundance were smaller and more variable, and no statistically supported differences among treatments were detected. Similarly, *Ae. aegypti* showed no consistent or statistically supported treatment effects. Species-specific LRR trajectories in fall reflected these patterns, with *Cx. quinquefasciatus* showing consistently negative values during treatment weeks (weeks 3–5) under spray treatments, indicating reduced abundance relative to controls. In contrast, LRR values for *Cx. tarsalis* and *Ae. aegypti* varied across weeks without a consistent directional trend (Supp. Fig. S1B).

Temporal dynamics of treatment-induced suppression: Temporal patterns of suppression differed between spring and fall treatments (Fig. 3). Weekly log response ratios (LRR) quantified changes in mosquito abundance in treated storm drains relative to contemporaneous controls, with more negative values indicating lower abundance in treated chambers.

In the spring trial, LRR values for both treatments were near zero during the pre-treatment period (weeks 1–2), indicating similar abundance between treated and control chambers prior to intervention (Fig. 3A). Following treatment initiation, LRR values declined under both treatments. The drip+spray treatment showed larger reductions during the treatment phase (weeks 3–5), with minimum LRR values of approximately -4 to -5 , while the drip-only treatment showed smaller reductions, with LRR values generally remaining between 0 and -2 . During the

post-treatment period (weeks 6–7), LRR values for both treatments approached zero, indicating convergence toward control abundance levels.

In the fall trial, LRR values declined following treatment initiation (Fig. 3B). Both spray-based treatments showed reductions during the treatment phase (weeks 3–5), with minimum LRR values of approximately -4 . LRR values increased during the post-treatment period (weeks 6–7), indicating a return toward control levels. Species-specific LRR patterns showed that reductions during treatment periods were primarily associated with *Cx. quinquefasciatus*, which exhibited negative LRR values during weeks 3–5 in both seasons (Supp. Fig. S1).

Occupancy and abundance as distinct outcome dimensions: Separating occupancy from abundance distinguishes how treatment effects were expressed across species (Fig. 4). Across both seasons, reductions in mosquito abundance were not consistently accompanied by declines in site occupancy. *Culex quinquefasciatus* maintained high occupancy across treatments and weeks (often ≥ 0.8), including under intervention treatments where abundance was reduced. Conditional abundance analyses (Supp. Fig. S2) show that, in both spring and fall, the number of mosquitoes per occupied site declined under intervention treatments, particularly for *Cx. quinquefasciatus*.

In the spring trial, conditional abundance under the drip+spray treatment was lower during treatment weeks (weeks 3–5) and increased in post-treatment weeks (weeks 6–7) (Supp. Fig. S2A). In the fall trial, conditional abundance under spray-based treatments remained lower relative to controls during treatment weeks (weeks 3–5) (Supp. Fig. S2B). Other taxa exhibited lower and more variable occupancy across treatments and weeks, consistent with lower overall detection frequencies. Together, these results indicate that treatment effects were expressed primarily as reductions in mosquito abundance within occupied sites, while occupancy remained relatively high for *Cx. quinquefasciatus*.

Phase-structured suppression and recovery of the dominant species: Phase-structured analyses focusing on *Cx. quinquefasciatus* (Fig. 5) show differences between spring and fall in how abundance changed across pre-treatment, treatment, and post-treatment periods.

In the spring trial, abundance during the treatment phase (weeks 3–5) was lower than pre-treatment levels (weeks 1–2) during intervention treatments, with the largest reductions observed under the drip+spray treatment (Fig. 5A). Following treatment cessation, post-treatment values (weeks 6–7) increased relative to treatment-phase levels and were similar to, or slightly higher than, pre-treatment levels. As a result, net change from pre- to post-treatment was small across treatments.

In the fall trial, abundance during the treatment phase was lower than pre-treatment levels across spray-based treatments (Fig. 5B). Post-treatment values increased relative to treatment-phase levels but remained lower than pre-treatment levels. Consequently, net change from pre-

to post-treatment remained negative across spray-based treatments. These phase-structured patterns are consistent with species-specific temporal dynamics. In fall, *Cx. quinquefasciatus* showed negative LRR values during treatment and post-treatment weeks (Supp. Fig. S1B), and conditional abundance remained lower during treatment weeks (Supp. Fig. S2B).

Discussion

Our results showed that habitat-targeted mosquito control suppresses populations primarily by reducing mosquito abundance within key refuges, rather than by complete exclusion from those sites. Given the relatively short treatment duration and the absence of direct effects on immature stages, the continued presence of mosquitoes in underground storm drain systems likely reflects ongoing emergence within chambers, in addition to residual adult presence. We did not distinguish newly emerged adults from resting adults using parity assessments or male genitalia rotation and therefore cannot fully separate ongoing emergence within storm drains from ongoing use of these habitats by older adults. Across seasons and treatments, the dominant effect was a reduction in mosquito abundance within underground storm drain systems. These habitats serve as important sites for oviposition, larval development, and adult refuge for urban *Culex* mosquitoes, and changes in mosquito abundance within them can influence mosquito activity beyond their physical boundaries. Suppression within storm drains was consistently accompanied by measurable, though smaller, reductions in mosquito abundance at nearby above-ground sites. This pattern indicated that habitat-level suppression was reflected in broader mosquito activity in the surrounding community, even if the translation from refuge suppression to above-ground activity was incomplete. Together, these findings indicated that spatial repellency and spray-based interventions were best evaluated through their effects on mosquito abundance, habitat function, and seasonal context, rather than by simple presence–absence outcomes.

Across both spring and fall, treatment effects were expressed primarily as reductions in abundance within occupied sites, rather than as loss of site occupancy (Fig. 4). The dominant species, *Cx. quinquefasciatus*, was detected at treated locations during many study weeks, though it was absent in some weeks during the treatment period. When present, mosquito numbers in treated sites were generally lower than in temporally matched control sites, as shown by conditional abundance analyses (Fig. 4; Supp. Fig. S2). This decoupling of occupancy and abundance aligns with ecological theory, which predicts that habitat quality can be degraded without forcing organisms to abandon sites entirely, particularly where alternative refuges are limited or spatially constrained (Pulliam 1988; Morris 2003). In mosquito systems, suppression of abundance reduces biting pressure, reproductive output, and transmission potential even when mosquitoes remain present (Killeen et al. 2003; Achee et al. 2012). Our results extend this framework by

showing that such suppression occurs within storm drain systems, a habitat type that disproportionately supports urban *Culex* populations. Continued site occupancy should therefore not be interpreted as evidence of treatment failure, particularly as public health goals typically center on reducing biting rates and pathogen transmission. Instead, abundance-based metrics more directly capture population-level consequences relevant to transmission risk.

The numerical dominance of *Cx. quinquefasciatus* strongly shaped community-level responses to the intervention. Because this species accounted for most captures across treatments and weeks, aggregate patterns largely reflect its response rather than uniform responses across taxa. Other species were consistently present but occurred at much lower numbers and showed more variable, often short-lived responses (Fig. 2; Supp. Fig. S1). Dominance-driven dynamics of this kind are well documented in ecological communities, where a small number of species account for most system-level variation (Hubbell 2011; Chase and Leibold 2009). In the context of mosquito control, this implies that substantial reductions in overall mosquito pressure can be achieved in systems dominated by a single vector species by targeting that dominant species, even when rarer taxa persist (Spielman et al. 2001; Kim et al. 2023). This pattern is particularly relevant because transmission risk is often driven disproportionately by a small number of abundant and competent vector species, rather than by overall mosquito richness or presence alone (Becker et al. 2010). In urban communities of southern California, *Culex* mosquitoes, especially *Cx. quinquefasciatus*, are primary vectors of West Nile and St. Louis encephalitis viruses, and their abundance is strongly linked to enzootic amplification and spillover risk (Becker et al. 2010; Reisen et al. 2008). Empirical and theoretical work has shown that reducing the abundance of dominant vectors can yield nonlinear reductions in transmission potential by lowering biting pressure, vector–host contact rates, and the effective reproductive number of pathogens, even when secondary species remain present (Snyder et al. 2020; Kwan et al. 2010). Consequently, changes in community composition alone can give a misleading impression of control efficacy if dominant, epidemiologically important species are not explicitly considered. Evaluating interventions therefore requires attention not only to species presence but also to species identity, relative abundance, and contribution to total vector pressure.

Treatment outcomes differed between spring and fall in both magnitude and persistence (Fig. 3; Fig. 5). In spring, the drip+spray treatment produced strong suppression during the middle of the treatment period (approximately week 4), followed by a pronounced rebound, with recovery to or above pre-treatment levels after treatments ended. Net change across phases was modest. In contrast, fall spray-based treatments, including both eugenol and peppermint sprays, produced stronger and more persistent suppression, with limited rebound and net declines relative to pre-treatment levels. These seasonal contrasts are consistent

with known differences in mosquito population dynamics across the annual cycle. Spring populations typically experience rising temperatures, faster development, and high intrinsic growth potential, conditions that facilitate rapid recovery following disturbance. Fall populations may have differed in demographic turnover, habitat use, or responsiveness to disturbance relative to spring populations, potentially contributing to the weaker rebound observed following treatment cessation (Reiner et al. 2013; Mordecai et al. 2019; Baafi and Hurford 2025; Whittaker et al. 2022). These results indicate that the timing of intervention relative to seasonal population trajectories plays a central role in shaping both short-term suppression and longer-term persistence.

Rebound following treatment cessation may also reflect changes in resource availability within treated habitats. By reducing mosquitoes within storm drain systems, interventions may temporarily free resources from habitat use and larval development. Reduced competition during treatment could allow these resources to accumulate, making habitats more favorable once treatment pressure is relaxed. Ecological theory predicts that reductions in density increase per capita resource availability and promote compensatory growth following disturbance (Tilman 1982; Murdoch et al. 2003). Such effects have been documented in insect populations following control or environmental perturbation, in which short-term suppression enhances subsequent population growth (Hastings 2004; Southwood et al. 1974). In mosquitoes, density-dependent competition can influence survival, development, and fecundity across life stages, although the strength and ecological expression of these processes vary substantially among habitat types and taxa (Juliano 2009; Alto et al. 2008). The partial rebound observed in spring, but not in fall (Fig. 5), is consistent with released resources interacting with seasonally favorable conditions to facilitate recovery. Although underground storm drain systems are not closed habitats and experience continuous environmental inputs and outflows, the effective availability of resources to developing mosquitoes is still expected to vary seasonally with temperature, water flow, and organic matter inputs. Although resource dynamics were not measured directly, this framework provides a plausible explanation for the observed seasonal asymmetry in rebound and underscores the importance of treatment duration, timing, and sequencing in systems with ongoing environmental turnover.

By measuring mosquito abundance above ground and within storm drain systems, this study assessed whether suppression within key refuges translated into broader suppression of mosquito activity. The intermediate declines observed above ground (Fig. 3; Fig. 5) indicate that reductions within refuges propagate outward, though not in a strictly proportional manner. This pattern is consistent with metapopulation and source–sink frameworks, in which certain habitats contribute disproportionately to population maintenance while remaining embedded within a broader network of connected sites (Hanski 1998; Leibold et al. 2004). Storm

drain systems likely represent a key habitat for *Cx. quinquefasciatus*, particularly in urban environments within arid regions such as the study area, where they may function as disproportionately important refuges relative to surrounding habitats. However, above-ground mosquito abundance is also shaped by dispersal, alternative refugia, and landscape heterogeneity. The partial correspondence observed here indicates that targeting storm drains can meaningfully reduce the pool of mosquitoes engaged in host-seeking activity, while acknowledging that no single habitat fully determines above-ground abundance.

Responses to intervention varied among species, with *Cx. quinquefasciatus* showing the most consistent and pronounced suppression across seasons (Fig. 2; Supp. Fig. S1). Other taxa displayed more heterogeneous and often short-lived responses, likely reflecting differences in habitat reliance, movement behavior, and sensitivity to intervention. Such interspecific variation is well documented in mosquito communities and has important implications for control in systems where multiple species coexist (Becker et al. 2010; Webb et al. 2021; Abella-Medrano et al. 2015; Reiskind et al. 2017). Habitat-targeted approaches are most effective against species that depend strongly on treated refuges, while more flexible or mobile species may require complementary strategies. Integrating habitat-focused suppression with interventions targeting other life stages or behaviors is necessary to achieve broad and durable control.

In summary, by separating abundance from occupancy and linking suppression in key habitats to changes in above-ground mosquito activity, this study provides an ecological framework for evaluating habitat-targeted mosquito control. Our findings show that spatial repellency and spray-based treatments applied to underground storm drain system (USDS) chambers produce abundance-mediated suppression of dominant mosquito populations, particularly *Cx. quinquefasciatus*, with outcomes shaped by seasonality and resource-mediated feedback. Rather than relying on binary measures of presence, these results emphasize the importance of evaluating control efficacy through changes in mosquito abundance, habitat function, and temporal dynamics.

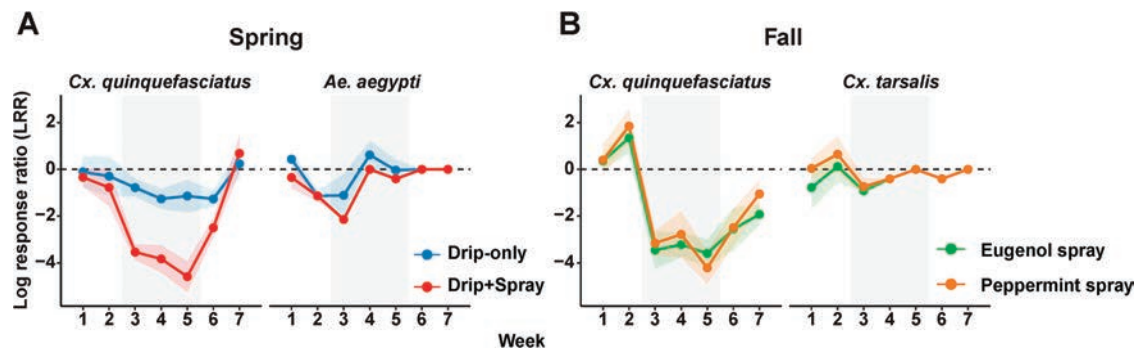
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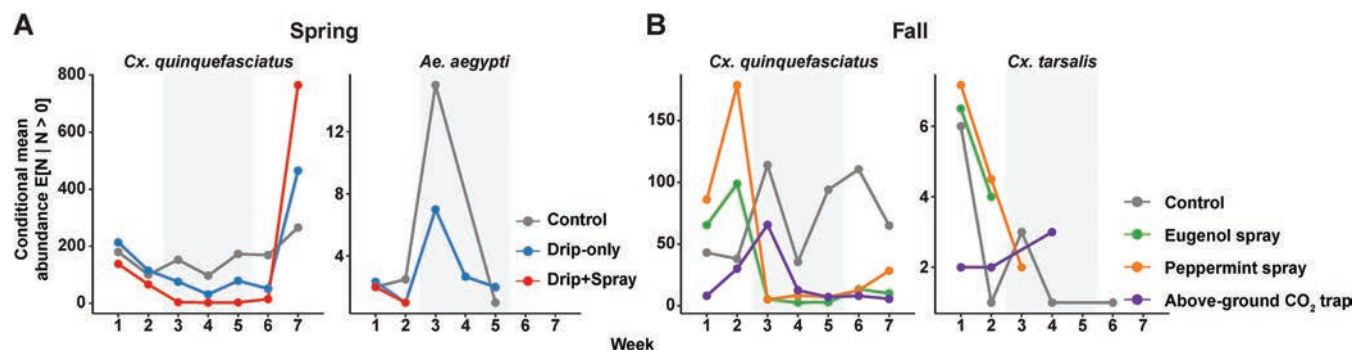
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Supplementary Figure S1.—Weekly species-specific log response ratios. Weekly treatment effects are shown as species-specific log response ratios (LRR) relative to contemporaneous control USDS units for the spring (A) and fall (B) trials, focusing on the two most abundant species in each season. LRR values were calculated as $\log[(\text{mean abundance in treated sites} + 1)/(\text{mean abundance in control sites for the same week} + 1)]$, such that plotted values represent log-transformed response ratios rather than raw abundance ratios. Lines represent weekly mean LRR values for each species-treatment combination, and shaded ribbons indicate $\pm$ SE. Panels are organized by species within each season. The horizontal dashed line denotes no difference relative to controls; values below zero indicate lower abundance in treated sites, and values above zero indicate higher abundance. Treatments were applied during weeks 3–5 (gray shading), with weeks 1–2 representing the pre-treatment period and weeks 6–7 the post-treatment period.



Supplementary Figure S2.—Conditional abundance across treatments and trials. Conditional mean abundance is shown for the dominant mosquito species in the spring (A) and fall (B) trials, calculated as the mean number of mosquitoes per USDS given presence ($E[N | N > 0]$). In spring (A), panels show *Cx. quinquefasciatus* and *Ae. aegypti*, comparing control, drip-only, and drip+spray treatments. In fall (B), panels show *Cx. quinquefasciatus* and *Cx. tarsalis*, comparing control, eugenol spray, peppermint spray, and above-ground CO₂ trap data. Lines represent weekly conditional means across weeks 1–7, with gray shading indicating the active treatment phase (weeks 3–5). By conditioning on mosquito presence within a USDS chamber, this metric isolates variation in local density within occupied sites from variation in site occupancy.

Using AI as a Subject-Matter Force Multiplier

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Abstract

Artificial Intelligence (AI) can function as a strategic asset by augmenting the capabilities of support staff within vector control operations. Integrating generative AI models, including Gemini, ChatGPT, Copilot, or Claude, into routine and complex workflows requires structured, expert-level prompts. Effective prompt architecture requires establishing a foundational persona and knowledge base, defining the target audience, imposing strict guardrails on output format, and executing a “pre-flight check”, where the AI is instructed to ask clarifying questions regarding missing data before proceeding.

Practical scientific and administrative applications include rapidly assessing the feasibility of multiplex RT-PCR assay designs, troubleshooting software like QGIS or Adobe Suite products, synthesizing data for biennial reports, and generating Python code to model mosquito abundance against environmental drivers. For complex projects, such as synthesizing dozens of source documents to develop a UAS Flight Operations and Safety Manual, multi-stage prompts maintain AI focus and facilitate progressive verification against hallucinations. Executing these advanced tasks effectively often requires investing in paid AI tiers, which can outperform free versions in complex reasoning, context retention, and data handling.

Operational integration of AI presents distinct challenges, including the risk of an AI acting as a subject-matter expert, while producing inaccurate outputs or mishandling sensitive data. Establishing organizational policy is critical. Protocols must govern the deployment of AI, control the upload of non-public data, and mandate the disclosure of AI-assisted analyses and recommendations that guide District actions. AI integration lowers technical barriers and accelerates data synthesis, but it does not replace human judgment. Strategic adoption of AI tools allows Districts to maintain operational advantages without sacrificing accuracy.

A digital framework for trustee oversight and board governance

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Abstract

A Digital Framework for Trustee Oversight and Board Governance Compliance presents a centralized application designed to streamline the management of trustees and board governance. The system enables keeping real-time attendance for board meetings, monitors trustee term limits and renewals, automated appointment/reappointment letters, and ensures adherence to regular compliance requirements such as AB1234 and AB1825. The application enhances transparency, accountability, and operational efficiency for organizations overseeing boards and trustees.

From clipboards to cloud: Modernizing information technology infrastructure at a mosquito and vector control district

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Introduction

Mosquito and vector control districts rely on digital systems for nearly every operational function, including surveillance data management, regulatory reporting, field operations, and public service request response. At the Marin/Sonoma Mosquito and Vector Control District (MSMVCD), infrastructure installed around 2010 had become a liability by 2022. An on-premises server was a single point of failure with no disaster recovery. Cat-5e cabling and aging switches constrained data throughput. End-of-life desktops required constant maintenance, and staff spent considerable time on manually reporting tasks that modern systems handle automatically. A cybersecurity incident made the operational risk concrete rather than theoretical. Broader pressures also played a role: cyber threats against public agencies were escalating, regulatory demands were increasing, and the public expected faster more transparent service. The goal of this project was to move MSMVCD off fragmented, on-premises legacy systems and onto a secure, cloud-based, mobile-enabled platform suited to present-day public health operations.

Methods

The project proceeded in phases. A formal cybersecurity audit was the starting point, producing a prioritized remediation roadmap and, critically, the documentation needed to pursue external funding. MSMVCD applied to the California Governor's Office of Emergency Services (CalOES) cybersecurity grant program and was awarded \$216,000, which made the accelerated timeline possible. Physical network infrastructure was upgraded from Cat-5e to Cat-6 cabling; Comcast Business Cable was replaced with fiber for symmetric, low-latency connectivity; and aging switches, access points, and the firewall were replaced with managed switches, updated access points, and a SonicWall (SonicWall Inc., www.sonicwall.com/products/firewalls) firewall. The on-premises server was retired and core operations were migrated to Microsoft 365 (www.microsoft.com/en/microsoft-365) and SharePoint (Microsoft Corporation, www.microsoft.com/en-us/microsoft-365/sharepoint/collaboration),

providing document management and version control, along with remote access and built-in redundancy. The district replaced reactive break-fix IT support with a managed services partnership offering 24/7 monitoring, endpoint protection, help desk coverage, and proactive maintenance. Internally, a Data Systems Specialist position was created. Staff cybersecurity training, phishing simulations, and penetration testing addressed the human side of security. The final major component was replacing MapVision 1.0 (Central UAS Technologies, www.centraluastech.com/products/mapvision-software/mapvision), in service since approximately 2010, with VeeMAC (VeeMac LLC, veemac.com), a platform built for mobile field data collection, real-time mapping, and integrated management of traps, treatments, and service requests.

Results and Discussion

Retiring the on-premises server removed the district's most significant single point of failure and established a cloud-based architecture with genuine redundancy and disaster recovery for the first time. Microsoft 365 and SharePoint gave staff secure remote access and document version control, a practical shift that changed how the district operates day to day, not just how data is stored. Moving to a managed IT partnership changed the district's security posture from reactive to proactive: threats are now identified and addressed before rather than after they become incidents. Staff training, including phishing simulations, extended that posture to the human side of the system, where most breaches actually originate. The deployment of VeeMAC was the most noticeable operational change, especially for field staff. Field technicians can now collect data on mobile devices and see surveillance and treatment data on live maps; trap monitoring, treatment records, and public service requests are managed on a single platform with automated quality checks rather than entered manually across separate systems. Overall, these changes moved MSMVCD from an organization running manual processes and aging hardware to one where field data flows from mobile collection through cloud processing

to operational dashboards in near real time. Several components remain in progress, and the full impact will not be apparent until those are complete.

Conclusions

MSMVCD's experience shows that full IT modernization is within reach for a small public agency, particularly when a security assessment leads to grant funding rather than a hardware shopping list. The district's path, audit first, fund through CalOES, migrate deliberately, and build internal capacity alongside

external infrastructure, produced durable improvements rather than a one-time equipment refresh. Districts facing similar legacy constraints should treat cybersecurity grant programs as a practical first step.

Acknowledgements

The author thanks the California Governor's Office of Emergency Services (CalOES) for the cybersecurity grant funding that made the accelerated timeline possible, and the staff and Board of Trustees of MSMVCD for their support throughout the project.

Connected for the Future: The journey of a districts' network infrastructure upgrade

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Abstract

The Solano County Mosquito Abatement District had limited internet service. This made it difficult to hold virtual meetings, share large files, and keep up with modern technology updates. As the District's work expanded and more devices needed to connect online, it became clear that a stronger, faster network was needed to support daily operations.

The District approved the network upgrade as a capital improvement project. Wi-Fi access points were placed throughout the property, giving coverage to about 90% of the five-acre facility. Completing the project wasn't easy, it required coordinating several vendors, and scheduling around ongoing office work. With teamwork and planning, the upgrade was completed successfully.

Today, the District can hold virtual meetings without interruption, update equipment such as electric trucks and drones remotely, and prepare to switch to an internet-based phone service. The faster connection also opens the door to future improvements such as a reliable camera security system and more cloud-based tools. This project has given the District the connectivity it needs to work efficiently and stay secure.

VectorSurv Mobile: take VectorSurv into the field

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Introduction

VectorSurv provides data management and analysis tools that allow mosquito and vector control agencies throughout California and the rest of the United States to make data-informed decisions. Because much of the work in mosquito and vector control takes place away from the office, there is value in using mobile devices to manage and use surveillance and control data. The VectorSurv team has received requests from partner agencies to consider developing specific mobile applications. To understand needs, we invited VectorSurv users to participate in a survey. Here, we describe key findings from the survey and preliminary plans for future mobile application development.

Methods

Users were invited to participate in the survey through several methods. There was an invitation in our quarterly newsletter, which is sent to all VectorSurv users and anyone else who has joined the newsletter mailing list. We also added a banner to the VectorSurv Gateway interface inviting VectorSurv users to complete the survey. Two weeks prior to closing the survey, we sent another email to all newsletter subscribers with another reminder to participate.

The survey was created in Qualtrics and was open for completion between 16 Sep and 31 Oct 2025. The survey included a series of questions intended to assess desire to use a mobile application, what functionality is needed, and what features should be prioritized. To assess whether a participant might use a mobile VectorSurv application, the first question asked whether the user already uses mobile devices for surveillance data management. A response of “no” was followed up with a question asking if the participant would consider using a VectorSurv mobile application, if it were to become available. An answer of “yes” to either of these questions was considered an indication that the participant might be interested in mobile applications.

For those users who indicated that they already used mobile devices for their surveillance, we asked some follow-up questions about the types of activities that would have the greatest potential for mobile applications, sizes of devices, preferred operating systems, and which equipment was already in use.

Several questions were designed to determine which functionality would be useful. To determine if offline functionality is necessary, the survey asked two questions. First, we asked, “How frequently do you work in an area with limited connectivity?” and provided options ranging from “Never” to “At least once a week”. Next, we asked “Is offline capability essential for VectorSurv’s mobile functionality to be useful to your agency?” We asked users to indicate their top three priorities for mobile development from a list of existing VectorSurv modules (sites, arthropod collections, arthropod pools, tick collections, tick pools, insecticide applications, integrated data view, service visits, calculators, and agency/user management) to understand which capabilities are desired. The survey also included open-ended questions where users could add comments or suggestions in free text to supplement their answers.

Results and Discussion

The survey received a total of 93 responses, although not every respondent answered every question, so denominators varied by question. Of the total responses, 36 (39%) were from VectorSurv partners in California. When asked about using mobile devices, we received 71 total responses. Of those, 31 (44%) already used mobile devices, 25 (35%) did not but would consider using a VectorSurv mobile application, and 15 (21%) did not and would not use a VectorSurv mobile application. Based on these responses, we estimated that 79% of VectorSurv users would potentially use a VectorSurv mobile application.

Of the users who indicated they already use mobile devices, 30 specified the type(s) of devices they use and how they use them. The responses indicated that 24 (80%) used mobile phones, 5 (17%) used small (< 8-inch) tablets, 18 (60%) used medium-sized (9- to 11-inch) tablets, and 4 (13%) used larger tablets. iOS was the most popular operating system, used by 26 (87%) of respondents, whereas 13 (43%) used Android devices and 3 (10%) used Microsoft Windows or other software. Current uses for mobile devices were reported as data visualization (n = 25; 83%), photo capture (n = 20; 67%), communication (n = 18; 60%), data management (n = 14; 47%), decision-making (n = 7; 23%), or other purposes (n = 1; 3%).

Most respondents reported working regularly in areas with limited connectivity [at least weekly (n = 21; 40%),

1-2 times per month (n = 9; 17%), less than once per month (n = 17; 33%), or never (n = 5; 10%]. One-third of respondents indicated that offline capability was essential (n = 17; 33%), while 27 (52%) indicated it was not, and 8 (15%) were unsure.

The most-desired capabilities for mobile application development were management of surveillance sites (n = 34; 76%), arthropod collections (n = 22; 49%), pesticide applications (n = 15; 33%), integrated data view (n = 15; 33%), service visits (n = 15; 33%), and arthropod pools (n = 14; 31%).

Overall, the survey indicated that providing offline mobile VectorSurv capabilities would add value for many agencies who use the system. In 2026, we will work with the CalSurv Steering Committee and others in

MVCAC to begin planning and development for these new features.

Acknowledgements

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Interactive dashboards for mosquitofish rearing operations and public outreach initiatives using Google Looker Studio

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Abstract

The use of mosquitofish (*Gambusia affinis*) as a biological control agent and the implementation of public outreach and education programs are essential strategies of Integrated Vector Management (IVM). By collecting relevant data on mosquitofish operations and public education efforts in Google Sheets, the Alameda County Mosquito Abatement District visualizes key metrics and trends in interactive dashboards using Google Looker Studio. The dashboards (1) support data accessibility and transparency by quantifying the efficiency of the District's mosquitofish rearing program and (2) promote an equity-based approach to public outreach efforts. This data modernization project has served as a catalyst for integrating dynamic dashboards to support evidence-based decision-making in IVM across both mosquitofish and public education initiatives.

The simple approach: Building a mobile drone treatment system

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Introduction

Modern mosquito control often faces significant logistical challenges when treating hard-to-reach habitats, particularly in marshland environments. Ground access to these areas is notoriously difficult and labor-intensive. Navigating through dense marshes can require several hours of travel, and movement frequently involves crossing multiple deep-water channels. Furthermore, there is a limited amount of chemical material that can be transported by ground machines or on foot. Traditional aerial operations address some of these access issues but typically require noisy, gas-powered pumps and cumbersome trailers to support flight operations. To address this operational gap, we developed a self-contained mobile drone treatment system designed for maximum agility, safety, and minimal environmental impact. The overall aim of this project was to demonstrate that a highly effective, “grab-and-go” drone system could be built without a complex fleet or massive budget, ultimately improving a district’s ability to efficiently treat inaccessible areas.

Methods

The mobile system was configured utilizing a PV40X drone, capable of a 40-pound dry larvicide payload and highly precise application rates. For the purposes of these operations, the drone was loaded with VectoPrime FG, a *Bacillus thuringiensis*, subsp. *Israelensis* (BTI) and (S)-methoprene formula, applied at a rate of 4 pounds per acre. The drone system was paired with a Ford F-150 Lightning electric truck. The truck’s Pro Power Onboard system was utilized to simultaneously run three drone chargers directly from the vehicle’s battery. To ensure safety and efficiency, the truck bed was outfitted with a camper shell and a slide-out bed tray to securely house the drone, Ground Control Station, batteries, and over 400 pounds of pesticides at waist level (Fig 1). To test the system’s modularity in extreme situations, the drone and essential charging equipment were hand-carried onto a landing craft to conduct spot treatments on Quimby (Fig 2), an island located on the far eastern border of Contra Costa County. It is a boat-access-only duck club

characterized by dense overgrowth where ground treatment is impossible.

Results and Discussion

The integrated electric vehicle and drone system successfully eliminated the need for a trailer-mounted generator, significantly reducing noise pollution and deployment times during standard operations on the mainland. During field applications, turnaround flights were remarkably efficient, averaging between 8 to 12 min per flight. Battery charging times were observed to be approximately 30 to 40 min. Although this charging duration is the same as when utilizing a traditional gas-powered generator, the F-150 Lightning provided a vastly quieter, cleaner, and more streamlined operation without the burden of hauling a trailer. Notably, the power draw from the F-150 Lightning was minimal, with the vehicle’s battery percentage dropping only about 1% for every three sets of drone batteries charged (Fig 3).

The ergonomic setup of the slide-out tray allowed crew members to safely deploy the drone and maintain continuous flight cycles, successfully treating up to



Figure 1.—The Ford F-150 Lightning truck bed outfitted with a camper shell and slide-out bed tray to safely house the drone and equipment.



Figure 2.—Satellite view of Quimby, an island that requires boat-only access.

10 acres per flight with minimal turnaround time on the ground. During the Quimby Island deployment, the modularity of the system proved critical. Because the equipment was not tethered to a traditional trailer setup, the team successfully ferried the drone and a portable generator via boat (Fig 4) to treat 50 inaccessible acres that would have otherwise gone untreated. Furthermore, real-time telemetry from the Ground Control Station confirmed accurate application rates of the VectoPrime FG, minimizing chemical drift and ensuring treatments were confined precisely to targeted mosquito breeding sites.

Conclusions

The combination of an industrial drone and an electric vehicle provided a highly replicable, scalable blueprint for mosquito control districts facing difficult habitat. By adopting this streamlined “grab-and-go” mobility, agencies can safely and cost-effectively treat previously inaccessible marshlands and island properties without the logistical headaches, extended travel times, or physical strain associated with traditional heavy equipment and ground treatments.



Figure 3.—Drone batteries charging efficiently from the Ford F-150 Lightning's Pro Power Onboard system.



Figure 4.—The PV40X drone and essential equipment loaded onto a landing craft for the Quimby Island deployment.

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Enhancing service request management with Survey123 and FieldSeeker integration

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Abstract

Because the San Joaquin Mosquito and Vector Control District has faced a surge in public service requests—particularly following the discovery of *Aedes aegypti* in 2019—our team sought ways to modernize and scale our request intake and response operations. Historically, all service requests were processed manually by front office staff, including those submitted online, which were emailed and re-entered into our GIS database hosted by Frontier Precision. This manual workflow was increasingly unsustainable and error-prone.

To address this, we implemented ESRI's Survey123, a customizable smart form tool that integrates seamlessly with our existing ESRI-based GIS environment and FieldSeeker application. Survey123 offers two flexible design methods: the web-based form builder for basic forms and Survey123 Connect—an Excel-driven tool—for advanced customization, including conditional logic and branding. We leveraged Survey123 Connect to tailor our service request form based on evolving District needs. For example, when residents frequently reported mosquitoes that were later identified as crane flies, we incorporated an image upload feature and educational prompting directly into the survey.

One key use of conditional logic in our form is enabling anonymous submissions: if residents choose to report anonymously, personal identification fields remain hidden, simplifying the user experience. This logic also was used to build internal-use-only versions of the form for management to preview and approve, demonstrating the tool's flexibility before public deployment.

On the backend, Frontier Precision engineers configured the Survey123 data to automatically push into FieldSeeker and assign field tasks to the supervisor responsible for that zone—no manual input required. To safeguard against missed requests, we added a workflow automation trigger that emails a summary of each new submission to office staff, who then cross-check with FieldSeeker logs. This ensures accuracy and accountability while minimizing staff workload.

Beyond service requests, Survey123 has helped expand our operational efficiency. We developed a QR code-enabled form for our swimming pool program that requires real-time photo uploads, eliminating the need for phone calls and ensuring current data.

Overall, Survey123 has significantly streamlined our public engagement and internal workflows, reducing manual data entry, minimizing response latency, and improving service accuracy. As public health challenges evolve seasonally, the flexibility of Survey123 allows us to quickly adapt and scale our solutions—empowering our District to better serve and protect our community.

Modernizing Mosquito Control: Program developments and UAS applications at Placer Mosquito and Vector Control District

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Abstract

The Placer Mosquito and Vector Control District (PMVCD) continues to advance its unmanned aircraft systems (UAS) program to enhance operational efficiency and support mosquito control efforts. Recent program developments include the integration of new data management tools, such as AirData™, to streamline flight logging, compliance, and mission planning, as well as the expansion of UAS applications for larvicide treatments. UAS technology has become a valuable tool for targeting hard-to-access habitats, improving treatment precision, and increasing operational efficiency compared to traditional ground-based methods. PMVCD is also evaluating new UAS ultra-low volume (ULV) spray systems to optimize droplet size, payload performance, and application accuracy for various adulticides. Together, these efforts demonstrate how UAS technology is becoming an increasingly important component of integrated vector management, supporting safer, more efficient, and data-driven mosquito control operations.

Closing the loop in mosquito control operations: Building a continuous learning system for vector control programs

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Introduction

Mosquito control produces results every day. Searches find or fail to locate sources. Treatments succeed, fail, or require follow-up. Those results matter, but in most systems they do not systematically improve future work. Mosquito control therefore functions largely as an open-loop system. Closing that loop offers a path to lower waste, target searches, and make control more effective.

Open-Loop and Closed-Loop Operations

The difference between open-loop and closed-loop operations is simple. In an open-loop system, actions are performed and recorded, but the results do not materially improve the next similar decision. Each event is handled largely on its own, with learning remaining informal, local, or dependent on individual memory. In a closed-loop system, outcomes from prior work are preserved in a form that affects later decisions. Over time, the system becomes better at reducing uncertainty, targeting effort, and choosing effective responses because it learns from what actually happened.

Example Operational Scenario and Discussion

This difference becomes clearer in a common district workflow. A district receives two kinds of signal at the same time: elevated trap counts and several public reports from the same neighborhood. In an open-loop system, those signals indicate that a problem exists, but they do not do much to narrow where the technician should search first. The technician begins with general area knowledge, visible conditions, site history, and professional judgment, then works outward through likely locations until the source is found. In a closed-loop system, those same

signals are combined with accumulated field outcomes from prior work across the district to produce a likelihood map showing where the source is most likely to be. Instead of beginning with a broad search, the technician begins with a ranked search area shaped by what similar patterns have previously revealed. As more district-wide outcomes are added over time, the search area becomes narrower and less effort is wasted on low-probability leads.

Once the technician reaches the source and classifies it, the difference continues. In an open-loop system, treatment is typically guided by standard practice, preseason assumptions, and technician judgment. In that approach, wasted product and ineffective treatment are not systematically recognized or addressed. In a closed-loop system, the technician receives a recommendation for product choice and application rate based on observed efficacy in comparable source types, environments, and operational conditions across space and time. The result is more effective control with less wasted pesticide.

Conclusions

Mosquito control improves when operational outcomes change later operational decisions. A closed-loop system gives districts a way to narrow search, guide treatment more effectively, reduce wasted effort, and reduce unnecessary pesticide use by learning from accumulated field results. Nidus (<https://github.com/Gleipnir-Technology/Nidus-iOS>) was built to support that operational model.

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Contra Costa Mosquito and Vector Control District's electrified journey

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Abstract

The Contra Costa Mosquito and Vector Control District (CCMVCD) is undertaking a multi-faceted journey toward Zero Carbon Energy, driven by the necessity of updating aging facilities and meeting evolving operational needs and compliance requirements. This presentation detailed the District's strategic approach to modernizing its infrastructure, a vital process given that the existing facilities date back to 1985 with a 1997 expansion. CCMVCD remains focused on incorporating emerging technologies to support both mission-critical work and long-term sustainability.

Internal operational needs and external regulatory shifts directly shaped the District's decisions, specifically in the successful adoption of solar panels to offset electrical consumption, the strategic integration of electric vehicles (EVs) into the fleet, and the simultaneous development of robust charging infrastructure to support this transition.

This comprehensive modernization effort includes facility upgrades such as energy-efficient lighting and upgraded HVAC systems, aligning with the District's commitment to utilize 100% renewable energy as a "Deep Green customer". Described were practical insights into the planning, challenges, and long-term operational benefits of executing an electrification strategy within a public health agency.

Data visualizations using the VectorSurv API

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Introduction

Vector surveillance programs generate substantial amounts of data that require effective visualization tools to support public health decision-making. Our presentation addressed the need for streamlined, programmatic access to VectorSurv data through its Application Programming Interface (API), enabling researchers and public health professionals to integrate surveillance data directly into automated analytical pipelines. By providing built-in R package integration and support for third-party API clients, VectorSurv aims to reduce barriers to data access and facilitate more efficient vector-borne disease surveillance and reporting.

Methods

VectorSurv offers API-based methods that can be used to automate the integration of real-time VectorSurv data into visualizations or analyses in external software such as R, Microsoft PowerBI, or Tableau. The `vectorsurvR` package offers a simple way to connect to the VectorSurv API and pre-built visualization templates, and standard HTTP clients which provide the ability for custom requests. Suggestions were offered about what information to include when communicating with the VectorSurv team for help with API requests.

Results and Discussion

The VectorSurv API and the `vectorsurvR` package provide options for VectorSurv users to visualize and

analyze their data. The built-in R templates available in `vectorsurvR` significantly reduce the effort required for common analysis or visualization tasks while maintaining flexibility for customization. The VectorSurv API provides options for users to access data from other programming environments. When contacting the VectorSurv team regarding API issues, users should include Agency ID, the exact URL accessed, and the request body (minus any token or password strings) to streamline communication and to resolve any questions. The availability of comprehensive documentation at <https://docs.api.vectorsurv.org> and ongoing email support at help@vectorsurv.org helps users to resolve technical challenges.

Acknowledgements

We thank our partners, especially the member agencies of the Mosquito and Vector Control Association of California and the California Department of Public Health, for their many essential and long-standing contributions to VectorSurv's mission to strengthen the evidence basis for vector-borne disease control. During 2025, VectorSurv's work was supported primarily by funding from the State of California and also by partial support from cooperative agreement 6NU50CK000632 to the American Mosquito Control Association from the Centers for Disease Control and Prevention.

Leveraging “Ally” — A Voice AI system to enhance operational efficiency and community response during *Aedes aegypti* expansion in Alameda County

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Abstract

Mosquito abatement districts play a critical role in safeguarding public health through vector surveillance, control operations, and community engagement. In 2025, the Alameda County Mosquito Abatement District (ACMAD) faced a significant operational challenge with the statewide expansion of *Aedes aegypti*, an invasive mosquito species capable of transmitting many viruses. The emergence led to heightened service demand, elevated public concern, and a greater requirement for swift and precise communication on a large scale.

To address these pressures, ACMAD deployed “Ally,” an AI-powered virtual assistant designed to enhance operational efficiency and improve public interaction. Ally operates across two primary channels: as a Voice AI system handling inbound telephone calls and as a web-based chatbot accessible through ACMAD’s website. The system delivers real-time, conversational responses to resident inquiries regarding mosquito control services, prevention strategies, and seasonal programs.

A distinguishing feature of Ally is its direct integration with ACMAD’s Work Order Management System. This integration enables the system to autonomously collect service request data, generate work orders, and guide residents through reporting potential mosquito sources. By combining natural language interaction with backend operational workflows, Ally reduces reliance on staff for routine interactions while maintaining consistent and accurate information delivery.

A major application of Ally during the 2025 season was its support of ACMAD’s Pool Projects initiative, which targets neglected swimming pools that serve as high-risk mosquito habitats. Ally assisted residents by gathering location details, facilitating inspection scheduling, and providing information on treatment processes and timelines. This capability proved particularly valuable during peak demand periods associated with *Ae. aegypti* activity, when rapid identification and mitigation were essential.

Preliminary outcomes indicate that Ally contributed to improved service coordination, reduced response times, and enhanced resident satisfaction. The system enabled ACMAD to scale communication efforts without a proportional increase in staffing, allowing field and operational personnel to focus on higher-priority vector control activities. Additionally, Ally’s consistent messaging supported public education efforts, reinforcing best practices for source reduction and community participation.

The deployment of Ally demonstrated the potential of AI-driven voice and conversational systems to modernize public health operations. By integrating communication, data collection, and workflow automation, such systems can increase organizational resilience and responsiveness during emerging vector threats. ACMAD’s experience provides a practical and replicable model for other mosquito abatement districts and public health agencies seeking to leverage artificial intelligence to meet growing service demands.

Where to Apply? Selecting locations for control efforts for *Aedes aegypti* in the Coachella Valley

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Abstract

Aedes aegypti has been routinely detected in California since 2011 and in the Coachella Valley since 2016. Although the Coachella Valley Mosquito and Vector Control District was created in 1928 and has been working to actively reduce mosquito populations and the viruses that they transmit since 1955, the establishment of *Ae. aegypti* has greatly impacted the routine work of the District through increased mosquito bites reported by residents, increased traps and mosquito collections, and increased mosquito treatments. The local transmission of dengue virus in nearby cities of Phoenix, Pasadena, and Long Beach further highlights the challenges that this species brings where residents a decade prior often questioned the need for mosquito control.

Since 2016, the District had made a series of large area larvicide applications to neighborhoods to control *Ae. aegypti*. Recent improvements to the planning process have resulted in considering more than just the mosquito population and service requests in deciding which neighborhoods to conduct these applications. Herein, we review our current efforts, highlight lessons learned in 2025 from comparisons of products and rates, and discuss plans for future control efforts.

Optimizing the diagnostic dose for the CDC bottle bioassay

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Introduction

Adulticides remain a critical component of integrated vector management, and resistance monitoring is essential for maintaining their effectiveness. The CDC bottle bioassay (BBA) is widely used to evaluate mosquito susceptibility, but interpretation depends on the use of an appropriate diagnostic dose. Recommended diagnostic doses have changed substantially over time (Brogdon and McAllister 1998, Peterson 2004, Mosquito and Vector Control Association of California 2016, Centers for Disease Control and Prevention 2024), including recent reductions for deltamethrin and naled. In addition, methodological variations among programs further complicates interpretation. Because the diagnostic dose is defined as the lowest dose producing complete mortality of susceptible mosquitoes within a specified time, differences in dose selection may influence conclusions regarding susceptibility. The objective of this study was to evaluate the impact of diagnostic dose selection on BBA outcomes and to identify an optimal diagnostic dose for deltamethrin using both traditional time-mortality criteria and dose-response modeling.

Methods

Bottle bioassays were conducted using technical grade deltamethrin (99.5% purity) with age matched susceptible reference colonies of *Culex quinquefasciatus*, including CQ1 (McAbee et al. 2004) and Sebring (Kent et al. 2010). For each dose, four replicates of 25 adult females were tested along with an acetone control. The tested doses were 0.75, 11.0, 15.0, 18.5, 20.0, 22.0, 26.0, 30.0 and 40.0 μg per bottle, and mortality was recorded at 5 min intervals for up to 2 h. Traditional time-mortality criteria were used to identify candidate diagnostic doses based on achieving 100% mortality within 30–60 min. In parallel, dose-response relationships at 45 min were modeled using bias-reduced binomial regression to address complete or quasi-complete separation and to obtain stable estimates at high mortality levels. Effective doses (ED90, ED99, and ED99.9) and associated 95% confidence intervals were estimated from the fitted models to identify doses producing near-complete mortality.

Results and Discussion

Time-mortality analyses indicated that the currently recommended CDC diagnostic dose of 0.75 μg per bottle

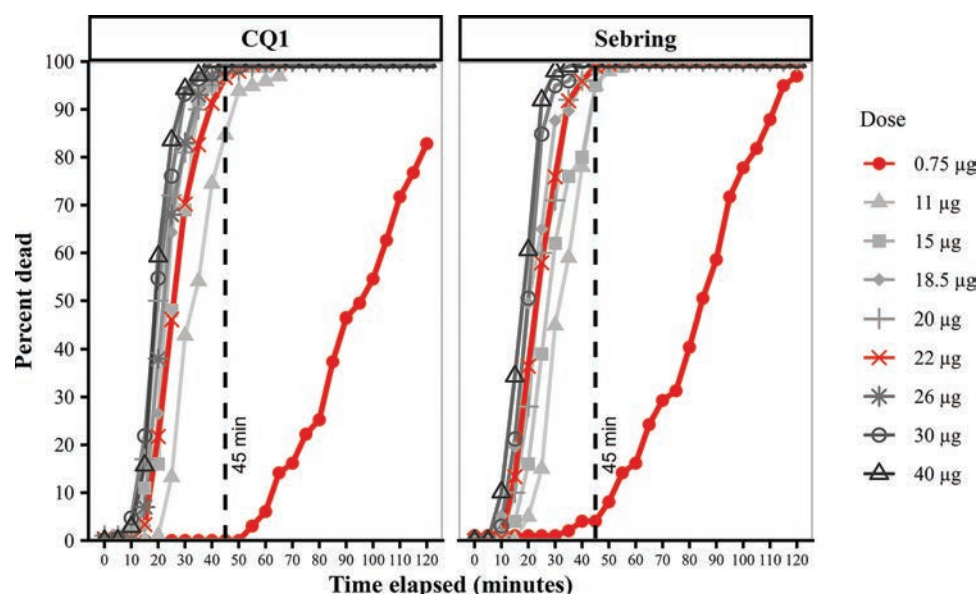


Figure 1.—Time–mortality curves from CDC bottle bioassays for susceptible *Culex quinquefasciatus* strains (CQ1 and Sebring) exposed to multiple concentrations of deltamethrin. Points represent observed mortality and lines connect mean percent mortality across replicates. The vertical dashed line indicates the 45 min diagnostic time. The SYMVCD diagnostic dose (22 μg per bottle) and the CDC-recommended dose (0.75 μg per bottle) are highlighted in red.

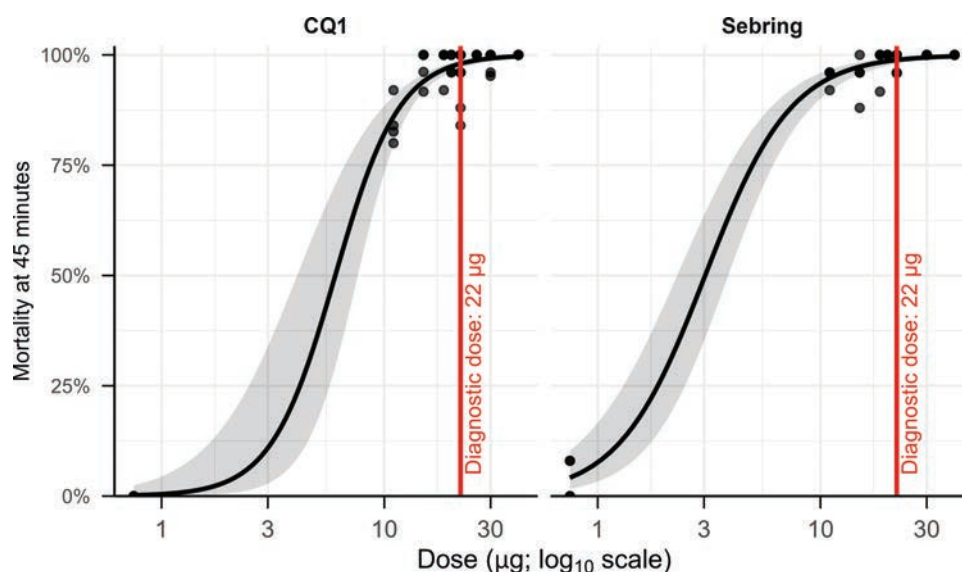


Figure 2.—Dose–response relationships from CDC bottle bioassays for susceptible *Culex quinquefasciatus* strains (CQ1 and Sebring) exposed to deltamethrin. Points represent observed mortality at 45 min and lines show fitted dose–response curves with 95% confidence intervals. The vertical red line indicates the diagnostic dose (22 µg per bottle), which corresponds to near-complete mortality for both strains.

did not achieve complete mortality within the expected time frame for either mosquito strain under our laboratory conditions (Fig. 1). Mortality increased with dose, but traditional approaches did not clearly resolve a single optimal threshold. Dose-response modeling provided improved resolution, with a steep increase in mortality across the tested dose range. For CQ1 mosquitoes, the estimated ED₉₉ at 45 min was 27.7 µg per bottle (95% CI: 22.8–33.7 µg), and for the Sebring strain, the ED₉₉ was 23.5 µg per bottle (95% CI: 18.0–30.8 µg). Estimated ED_{99.9} values were slightly higher, but overlapped broadly with these intervals, reflecting the asymptotic nature of the dose-response curve near complete mortality (Fig. 2). The diagnostic dose currently used by SYMVCD (22 µg per bottle) fell within or near the ED₉₉ confidence intervals for both strains and produced near-complete mortality, with only occasional individual survivors observed. These findings indicated that lower diagnostic dose recommendations may, in some cases, result in measurable survival among otherwise susceptible populations. Additionally, even under controlled laboratory conditions, stochastic variation in mortality near the upper asymptote of the dose-response curve results in occasional survivors, reinforcing that diagnostic thresholds should be interpreted probabilistically rather than as absolute cutoffs.

Conclusions

A deltamethrin diagnostic dose of approximately 22 µg per bottle is appropriate for both susceptible reference strains of *Cx. quinquefasciatus* evaluated and provides a reliable operational benchmark for resistance monitoring. These results highlight the importance of evaluating diagnostic doses under local conditions, as variation in assay design,

mosquito populations, and environmental factors may influence outcomes. Incorporating dose-response modeling into assay development provides a quantitative framework for selecting diagnostic doses and may improve consistency and interpretability of BBA results across programs.

Acknowledgements

We thank laboratory and field staff at the Sacramento-Yolo Mosquito and Vector Control District for assistance with bioassays and data collection.

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All Wetlands are Not Alike: Integration of Traditional Ecological Knowledge (TEK) for wetland management, biodiversity, and vector control

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Introduction

Wetland ecosystems can either mitigate or exacerbate the risk of vector-borne disease transmission, depending on their ecological integrity and management practices. Our presentation examined how wetland ecosystem health determines whether these habitats function as beneficial assets or public health hazards and documented ongoing collaboration with indigenous traditional ecological knowledge (TEK) practitioners to transfer critical knowledge and integrate western scientific approaches for ecosystem health monitoring.

California's ecological constitution has changed dramatically over the past 200 years due to colonization, the displacement of indigenous peoples, and the disruption of their land management practices (Whipple et al. 2012). These landscapes did not degrade on their own; they reflect the loss of generations of deliberate stewardship. This is reflected in a 95% loss of wetland habitat throughout the central valley and southern regions over the past 200 years, driven by agricultural expansion and urban development. Current wetlands, surrounded by agricultural environments, suffer from nutrient influx that drives bacterial overgrowth, eutrophication, reduced biodiversity, and increased mosquito abundance while promoting insecticide resistance (Gustafson and Wang 2002, Mercer et al. 2005, Buxton et al. 2020, Boerlijst et al. 2023). This results in a reduction in overall biodiversity, disruption of ecosystem stability, and loss of predatory species that can regulate vector populations. In addition, the presence of insecticides and other toxic compounds in runoff may also negatively affect macroinvertebrate abundance and can drive insecticide resistance in mosquitoes (Porretta et al. 2022, Lee et al. 2023). These conditions have led Western cultures to view wetlands as disease-associated 'wastelands,' thereby prioritizing their conversion for agricultural or urban/suburban development in the absence of knowledge of the long-term ecological and public health benefits that properly managed wetlands can provide (Vileisis 1999).

Prior to colonization, indigenous communities maintained California's pervasive wetland ecosystems for millennia

through sophisticated management practices developed over many generations, fostering a culture of subsistence that depended on ecosystem health, stability, productivity, and clean water. These practices ensured plant health and maintained biodiversity. This was, in part, accomplished through cultural burning practices, which eliminated overgrown plant material, stimulated growth, removed invasive plants, and generated biochar. Biochar provides ecological benefits, including carbon sequestration, water filtration, and enhanced microbial diversity (Humberto Blanco-Canqui 2019, Pereira et al. 2021). In addition, the deep knowledge of native plant biology and ecology facilitated planting and maintenance techniques to prevent overgrowth and optimize plant health (Mucioki et al. 2026). Native communities harvested wetland reeds and other plant materials in substantial quantities for food, structures, and craft, resulting in the cycling of nutrients and organic matter through the system. The severing of these practices left soils without the traditional replenishment; as intensive, repeated cultivation became the norm, declining fertility drove growing reliance on synthetic fertilizers, whose runoff now contributes to the nutrient influx degrading remaining wetlands.

Methods

Our outreach efforts have focused on the Cache Creek watershed on the homelands of the Yocha Dehe Wintun Nation of Patwin Indians, including partnerships with independent Patwin/Wintun cultural practitioners, knowledge bearers, and fire practitioners. We have developed community workshops on traditional burning and wetland management techniques that incorporate education on wetland ecology/biology, vector biology, the cultural and ecological value of wetlands, and collaborative research in integrating western scientific monitoring methods with traditional knowledge systems. Participants also practice native plant identification and cultural uses; harvesting and preparation techniques for wetland plant-based materials; weaving and ropemaking; and traditional methods for preparing for, starting, and managing cultural burns. The knowledge and techniques taught in the workshop are used

to create floating mats that serve as platforms on which live, interwoven wetland plant roots can establish footholds in areas requiring revegetation.

Results and Discussion

Well-maintained and biodiverse wetland ecosystems provide multiple mechanisms to reduce vector-borne disease risk. Biodiverse wetlands reduce disease risk through dilution effects (abundant non-permissive vertebrate hosts), interspecies competition that limits vector expansion, and robust predator communities, including copepods, dragonflies, and backswimmers that regulate mosquito populations (Juliano 2009, Rey et al. 2012, Benelli et al. 2016, Keesing and Ostfeld 2021). This challenges a pervasive paradigm that views wetlands primarily as vector production sites requiring either drainage or intensive management via chemical suppression. Instead, the evidence indicates that ecosystem integrity, not wetland presence, determines disease risk, and healthy, biodiverse wetlands provide net protective benefits against vector-borne disease transmission, along with essential ecosystem services addressing critical environmental issues, including water purification, flood mitigation, aquifer recharge, carbon sequestration, biodiversity enhancement, temperature regulation, and bioremediation of toxins such as heavy metals (Duffy and Kahara 2011). Research shows that restoration activities guided by Indigenous traditional ecological management practices offer superior outcomes compared to contemporary drainage and mechanical management approaches (Méndez-Toribio et al. 2021, Souther et al. 2023, Walker et al. 2026). Successful implementation of TEK-informed wetland management requires the establishment of genuine, trusted, and long-lasting relationships and collaboration with indigenous practitioners and communities (Walters et al. 2020).

Conclusion

This work provides strong support for integrating ecosystem-level processes and the wisdom embedded in Traditional Ecological Knowledge into vector control strategies. By shifting from a reductionist approach focused solely on vector elimination to a holistic understanding of ecosystem health, enhanced control outcomes can be achieved alongside the broader environmental and public health benefits of healthy wetland ecosystems.

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Thermal stress and pyrethroid resistance in the invasive mosquito, *Aedes aegypti*

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Abstract

The yellow-fever mosquito, *Aedes aegypti*, poses significant public health risks in California urban environments, where high summer temperatures coincide with increased biting, increasing the risk of dengue virus transmission. This study investigated the relationship between thermal stress and pyrethroid resistance in *Ae. aegypti* populations in Madera, CA. Using CDC bottle bioassays with CDC technical grade λ -cyhalothrin (10 $\mu\text{g/mL}$), three temperature-treated (18°C, 26°C, and 34°C) wild-type F0 female Madera (MAD) strain mosquito groups were compared to the ROCK laboratory susceptible strain. Time-mortality data revealed treated (26°C) MAD strain mosquitoes had the highest mortality rate 30 minutes post-exposure, inferring temperature treatment influences survivability. To bridge understanding of technical resistance and practical resistance in the field, an ultra-low volume spray (ULV) field trial at three sites—one site per temperature treatment—was conducted with wild-type F0 MAD female *Ae. aegypti* and the susceptible ROCK strain using commercial grade Demand CS. All temperature treated MAD groups had an overall knockdown rate of $\sim 0\%$, 1-hour post-exposure and a knockdown rate of $\sim 3\%$, 12-hours post-exposure. This finding suggests that MAD strain mosquitoes are highly resistant to field application of Demand CS. Combined field and laboratory data demonstrated that laboratory-based bottle bioassays do not accurately predict real-world outcomes. Alive and dead mosquitoes were pooled from each assay and field trial for RNA extraction and transcriptomic analysis. Gene expression quantification for HSP70, HSP20, HSP83, CYP9J32, CYP9J28, CYP6M11, CYP6BB2, V410L, V1016I, and F1534C will provide insight into the transcriptomic response to pyrethroid exposure. The results from this study characterized the relationship between thermal stress and pyrethroid resistance. Identifying resistance markers in field populations will enable more targeted *Ae. aegypti* management in California and guide vector control strategies.

WALS treatment in San Diego cemeteries in response to elevated levels of West Nile Virus

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Abstract

In 2025, elevated levels of West Nile virus were detected by the San Diego County Vector Control Program (VCP) in *Culex quinquefasciatus* caught in and around a cluster of four cemeteries within a 1/2 mile radius in a densely populated community in the City of San Diego. Although WNV levels were below thresholds for adulticide treatment, we rapidly initiated inspections of nearly 15,000 potential breeding sites, such as flower vases and landscape drains on the cemeteries, of which 75% held water and 50% contained mosquito larvae. In addition, 47 additional site inspections were performed in adjoining neighborhoods. An expanded trapping and testing protocol with gravid and EVS traps was conducted weekly at all four cemeteries and adjacent neighborhoods within the 1/2 mile radius to determine possible sources of mosquitoes. Twenty-six staff were mobilized during the first two days to inspect, dump out water, and treat larval sources with granules of VectoMax[®] FG (Azelis, USA). However, it became apparent that additional treatments would be necessary until the cemetery could implement source reduction practices to reduce mosquitoes on their own and that a more efficient treatment method would be needed. Therefore, for subsequent treatments, VCP utilized a buffalo turbine to apply VectoBac[®] WDG (Azelis, USA) to treat breeding sources throughout the cemeteries. Using the Wide Area Larvicide Spray (WALS) method, fifty acres were treated in less than 20 min by 3 staff. Mosquito trap yields decreased from an average of 41 to 4 mosquitoes per trap-night. Furthermore, larval assays demonstrated 97% efficacy of the treatment. No additional WNV positive mosquito pools were detected in the area over the course of the season, and costs were reduced by over 80% by using the WALS treatment versus hand treatments. To reduce the risk of WNV in future years, VCP is working with cemeteries to implement their own integrated vector management programs and to facilitate the use of WALS in these settings.

Evaluating seasonal efficacy of aerial and ground-based adulticiding in Yolo County, California

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Abstract

Rice fields are among the most productive mosquito habitats in California's Central Valley, and with more than 30,000 acres in Yolo County alone, they serve as a major source of mosquitoes that are important vectors of West Nile virus and other arboviruses. Effective management of these habitats is critical for public health protection and the success of regional mosquito control efforts. Assessing the efficacy of control operations can be difficult because of the lack of untreated control sites and the amount of trapping necessary to effectively measure abundance trends.

For the current project, the Sacramento–Yolo Mosquito and Vector Control District conducted a comparative field study to evaluate the seasonal efficacy of operational adulticide applications in rice field habitats from June through October 2025. Two sites were selected: one routinely treated with aerial and truck-mounted adulticiding operations using a variety of active ingredients (Dibrom, Naled, EverGreen525, Pyronyl 525, and Deltagard/Deltamethrin formulations), and a reference site that received no, or limited, adulticide treatments during the study period. At each site, 20 adult mosquito CO₂ traps were operated at night continuously Monday through Friday from 10 Jun through 10 Oct 2025 (18 weeks), providing high-resolution data on mosquito abundance and species composition throughout the season. A total of 25 unique adulticide treatments (17 aerial and 8 ground-based applications) were evaluated. Environmental variables including temperature, wind speed, and humidity were recorded at each collection.

Species composition was dominated by *Culex tarsalis* (83.6%), followed by *Anopheles freeborni* (9.5%), *Aedes melanimon* (4.5%), and other species (2.0%). A negative binomial regression model controlling for weather, site effects, spatial dynamics, and seasonality was used to assess percent reduction in mosquito abundance following adulticide application, examining abundance on the day before (–1), day of (0), and 1–2 days after treatment. No detectable immediate reduction in mosquito abundance was observed on the day of treatment relative to the control area. Subsequent post-treatment analysis revealed approximately 28–34% reduction in mosquito abundance in the treated area, although these reductions were not statistically significant. The lack of statistical significance indicates limited detectability attributable to temporal and spatial dynamics in rice field systems, rather than operational failure. These findings provide quantitative assessment of adulticide efficacy under field conditions and contribute to evidence-based evaluation of control program effectiveness. Additional research employing novel age-grading methodologies such as Surface Enhanced Raman Spectroscopy for *Culex* spp. could be used to complement traditional surveillance-based efficacy assessment.

Mosquito production sites – Tackling the threat of neglected pools

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Problem

Due to the year-round activity of mosquitoes, the Coachella Valley Mosquito and Vector Control District (District) dedicate resources to control mosquito populations in urban settings. Neglected swimming pools represent a significant larval source for several mosquito species able to transmit arboviruses to residents of the Coachella Valley. As a District, it is imperative that we address and manage these important sites while navigating and adhering to the legalities associated with private property and individual rights to privacy. The mission of the District is to reduce and control mosquito sources and in turn protect public health. In the present discourse, effective means to mitigate the adverse conditions created by neglected pools were identified and actionable solutions brought forth by streamlining the appropriate District responses.

Solutions

Neglected pools are an important source for urban mosquitoes that are identified through aerial imagery, service requests and information provided by code enforcement agencies. To access these sites on private property, there is a legal process in place and for that we utilize a warrant to gain entry to private properties to inspect neglected pools. Depending on the status of the home [occupied or vacant], a notice(s) is posted before executing the warrant to give the homeowner or resident advanced notice and the opportunity to communicate with the District. If no response is received, a warrant is executed and access is gained, the neglected pool is inspected and treated accordingly based on the technician's findings. Water depth, time of year and future plans for the neglected pool are considered when choosing the most effective treatment options.

When feasible, the District utilizes a two-tier approach to getting a neglected pool producing mosquitoes under control. At our disposal is an arsenal of chemicals and their various formulations along with biological control using *Gambusia affinis*, commonly known as mosquitofish. Mosquitofish have been reported to eat between 15 and 65 mosquito larvae in 30 minutes. One fish ate 168 larvae in 8 hours by itself during observations by the District. By

pairing a quick kill surfactant along with mosquitofish, a neglected pool can come under control quickly and stay under control for a prolonged period.

Water depth and condition determine whether an application of mosquitofish is appropriate. If the water is too low or too polluted, the mosquitofish will not survive (Fig. 1). Chemical treatments take into consideration debris, depth and accessibility to pool.

If a pool is completely dry, the homeowner has the option to enroll in the District's pool program. This allows homeowners to submit photo proof of the pool's condition during regular intervals throughout the year to avoid a technician visit to their property. The pool program is managed through the Zingle application (<https://app.zingle.me>), which enables the District to organize, sort and classify pools enrolled in the program. This saves the District time and resources.



Figure 1.—A neglected pool on private property approximately 50% full of stagnant green water was found to be producing mosquitoes.

Conclusions

Neglected pools must be kept at the forefront of any District's response to tackling mosquito production within their service area. Neglected pools require additional effort to address but the reduction in mosquito numbers merits the investment of time and resources. Mosquitofish are an

invaluable means of biological control and when combined with a chemical application can bring a neglected pool under control quickly. Direct communication with homeowners and periodic check-ins through the Zingle application expedites follow up and facilitates the allocation of resources where appropriate. Neglected pools present a challenge to any District but with the proper attention can be managed effectively.

How to “Level Up” outreach for *Aedes aegypti* without missing the mark

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Introduction

Since 2022, the Contra Costa Mosquito and Vector Control District (District) has detected *Aedes aegypti* within Contra Costa County. With the initial detection, the District’s Public Affairs staff were tasked with educating Contra Costa County residents on the fact this mosquito typically bites during the day, it can transmit viruses that can lead to dangerous diseases, and providing tips on what residents could do to reduce the risk of these mosquitoes. While conducting that and subsequent outreach, the Public Affairs staff soon learned what worked in one year, may not work as well in subsequent years, so keeping track of outreach in real time is not only beneficial, it’s important to truly educate the public.

First *Aedes aegypti* Detections and Outreach

When the District first detected *Ae. aegypti* in late 2022, it was in a limited area of one neighborhood within the city of Martinez. The Public Affairs staff worked to educate Martinez residents through e-newsletters, news releases, social media, public events and presentations. The District was able to control the invasive mosquitoes in Martinez and while public outreach on *Ae. aegypti* continued, it was no longer specifically focused on Martinez.

Two years later, two more detections of these invasive mosquitoes were made in late 2024 that revealed the presence of this species in other areas of Contra Costa County prompting increased efforts to control and educate the public. One detection was potentially an early introduction at a cemetery where control was successful; however, the other detection that occurred while a District employee was providing a residential service, revealed a more widespread number of *Ae. aegypti* within the city of Antioch. As this was in the fall of 2024, Public Affairs staff members once again published e-newsletters, news releases and social media and participated in public events and presentations to get the word out to the people of Antioch. Staff also planned a community meeting at a local park to share with residents what the District was doing to try to control these invasive mosquitoes and what resident can do to reduce the risk on their own properties. The event at the local park was well attended by more than 60 local

residents, and provided an opportunity to share factual information to the residents of Antioch.

How We Missed the Mark

In 2025, the District detected *Ae. aegypti* in Antioch again, this time earlier in the year, giving the Public Affairs staff more time to plan. After the successful community meeting in 2024, we planned to have another one later in 2025. In the meantime, we distributed printed fliers to schools in Antioch, postcards to residents, e-newsletters, news releases, social media, and participated in the public events and provided informational presentations. In the summer of 2025 we chose two parks in Antioch for community meetings and invited residents to have their questions answered by District employees. Although we anticipated exceeding the 60+ attendees from the year before, crowds did not come. Instead, by the end of the events, there were no more than 22 citizens who came to have questions answered at the two parks. We were surprised and disappointed. Then we looked back at the outreach we’d already conducted.

We’d distributed bi-lingual fliers to every public-school student in Antioch, we sent informational postcards with a QR code to District information to every resident in the detection zone, we provided updated information in the e-newsletters, real time information in targeted social media messages sent directly to the residents of Antioch, participated in the Contra Costa County Fair which is in Antioch and provided presentations to the City Council and community organizations. We soon realized, we’d already provided the information and likely answered the majority of questions through all of the outreach in the months prior to the community meetings.

How to Avoid Missing the Mark

Following the disappointing turnout for the 2025 community meetings, we looked into options to track current outreach when planning future outreach, and found a real-time Outreach Tracker from the Mosquito and Vector Control Association of California that, when used by Public Affairs staff, kept track of the outreach done in real time and kept track of which residents were reached. In 2026, we plan to avoid missing the mark of unreasonable expectations by using

the real time Outreach Tracker. We also plan to communicate more effectively as a Public Affairs Team to make sure we have input from all members and that each of us are using the Tracker to make sure our outreach provides quality information and does not call on residents to attend an event to have questions answered, when we've already answered their questions through thoughtful and informative outreach.

Acknowledgements

I'd like to thank Andrew Pierce, the District's Public Information and Technology Officer; and Christine Widger, the District's Customer Service Specialist for their efforts as members of the Public Affairs Team and their help with outreach each year.

From in-person participation to on-camera ready: The importance of always being prepared to be recorded at outreach events

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Introduction

The public affairs staff at the Contra Costa Mosquito and Vector Control District (District) work diligently to communicate important information to Contra Costa County residents specific to the District's programs and services, and to answer inquiries made by the public, local news media, and constituents. Outreach efforts are provided by way of written articles, news releases, social media posts, public speaking engagements, and through participation by in-person events throughout the county. In the past year, District staff have observed that members of the public (including local news reporters and public officials) who attend in-person events are increasingly using their phones to record video of event personnel. It quickly became apparent that being prepared for the possibility of being on camera while working at an in-person outreach event was essential, not only to alleviate the stress among employees that can occur when being recorded on video, but because people who are recording content often subsequently post their videos to their personal or professional social media accounts.

That was then, this is now

When preparing for one of the Districts in-person outreach events, the focus on logistical protocols and supplies needed for a successful event have remained constant. Specifically, the physical items necessary for the District's event booth included a canopy, table and chair, literature, live specimens, and branded giveaway items. Another "pre-event" staple included reminding staff beforehand to be engaging, polite, and eager to answer questions. Although visitors occasionally photographed display items, such as an emergence jar containing live larvae and adult mosquitoes, the protocols had to be updated to adapt to the changing realities of staffing a District event booth. Added to the original logistics protocols, a new checklist centered on the expectation that attendees, particularly local news media or public officials, will record video of District staff and possibly ask questions. For some District employees, an impromptu interview could be disconcerting. The importance of being prepared to be recorded on video while working at an in-person outreach event is paramount now, more than ever.

Steps to ensure success

When preparing for a District in-person outreach event, District employees must now make sure they follow an updated protocol to be prepared for members of the public and the potential for being recorded on video. These guidelines include proper attire, updated talking points and a reminder to remain professional and courteous while addressing members of the public, including anyone who might record video or ask questions during the event. Attire must represent the District properly, such as wearing a District logo polo shirt or sweater, clean socks with comfortable shoes. If employees from the District's Operations Department are working an event, they must wear their District uniforms, and their identification badge, so that members of the public can readily recognize them as District employees, both at the event and while the employees are providing District services elsewhere.

Prior to the event, the public affairs director should meet with District supervisors to confirm any ongoing issues in the city where the outreach event is taking place. The information is used to better inform the talking points that will be used during the event. Typical event talking points include information on the District's mission statement, the District's services and the fact that Contra Costa County residents already pay for the District's services through property taxes and benefit assessments, so there is no additional charge for residential services. In addition, updated talking points are now provided so each District employee is prepared if a member of the news media, a resident, or a city or county official begins recording video (Fig. 1). In that case, the employee would be prepared to answer questions with accurate, District-approved messages appropriate for recording. Among the talking points, the District employee also should be instructed that, if the opportunity arises, the employee may enquire about the type of story a member of the news media is working on, or offer to help a city or county official or a member of the public with their video content. While providing information, the District employee must remain professional and calm when answering any questions as this reflects positively on the District and would likely leave the person recording the video with a favorable impression of the District.



Figure 1.—Andrew Pierce, Public Information and Technology Officer, conducting an interview with a local community television station during an in-person outreach event.

Once the in-person outreach event has concluded and the District employee has returned to the District, the employee should work with the District’s public affairs team to look up the video and, if possible, record the video to review the District employee’s answers. If the District employee handled the interview and overall video situation well, including sharing District talking points properly, then the video could serve as a training video for other District employees who may be in the same situation in the future.

Conclusion

An important component of any Mosquito and Vector Control District is public relations through in-person community outreach events, which provide a valuable opportunity to share important District information with the public. District employees who work in-person outreach events represent the District well when they

appear professional, are prepared with the appropriate talking points, and answers to questions from members of the public, news media or public officials with confidence, accuracy, and professionalism. The added benefit for the District, is when the videos and interviews prove to be successful, because that can provide the District with the opportunity to reach more people within the District’s jurisdiction that can learn about the Districts’ important public health messages while gaining new knowledge of the District’s job to protect public health.

Acknowledgements

The author would like to express appreciation to the Contra Costa Mosquito and Vector Control District’s public affairs staff for their dedication to working in-person outreach events and their understanding of the importance regarding professionalism and enthusiasm pertaining to interactions with the public.

The Buzz behind the release: Public outreach strategies for a Sterile Insect Technique pilot program

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Introduction

Invasive *Aedes aegypti* continue to expand across California, posing a significant public health concern due to their ability to transmit viruses such as dengue, Zika, and chikungunya. Each year, travel-related cases of dengue are reported throughout the state, and more recently, local transmission of dengue has been documented in California, signaling a growing public health risk. As these trends continue, the need for innovative and effective mosquito control strategies becomes increasingly important. In response, the Sacramento-Yolo Mosquito and Vector Control District (District) launched a Insect Technique (SIT) pilot program in South Natomas in July 2025.

A critical component of this pilot program was the development and implementation of a strategic, comprehensive public outreach campaign designed to inform, educate, and build trust with residents. With an accelerated timeline from project approval to launch, effective communication was essential to ensure community understanding and acceptance of this innovative approach.

The current presentation highlights the outreach strategies used to support the SIT pilot project, key challenges encountered, and lessons learned that can be applied to future public health initiatives.

Methods

The District implemented a comprehensive, multi-faceted public information and outreach strategy to support the SIT pilot program, with an emphasis on transparency, community engagement, and building public trust. Given the accelerated timeline from project approval in April to program launch in July, outreach efforts were developed and deployed within a condensed timeframe, requiring close coordination across departments and with external partners.

Messaging focused on clearly explaining the science behind the release of non-biting male mosquitoes using simple, accessible language to ensure public understanding and reduce potential concerns. To maintain consistency, internal briefings and staff training were conducted so all

District personnel could effectively communicate key project information.

Key outreach components included the development of a dedicated project webpage, frequently asked questions, and bilingual educational materials. A door-to-door hanger was distributed to over 1,200 homes within and surrounding the pilot area. In addition, a targeted digital advertising campaign—including streaming television and radio, geofenced display ads, and direct email outreach—was implemented to reach residents within the project zone.

Community engagement remained a central focus, with presentations conducted for neighborhood associations and coordination with local elected officials to ensure awareness and support. Media relations also played a critical role, including the development of press materials, proactive pitching of stories, and coordination of a media event to mark the program launch.

Together, these integrated outreach efforts were designed to inform residents, address concerns, and build confidence in the implementation of this innovative vector control strategy.

Results and Discussion

The comprehensive outreach strategy successfully generated widespread awareness and understanding of the SIT pilot program within the target community. Proactive engagement with stakeholders and residents helped build trust and foster transparency, which was essential for introducing a novel mosquito control technique.

One of the primary challenges encountered was an unexpected early media release, which required rapid adaptation of communication efforts. Despite this, the District was able to quickly respond with clear and consistent messaging, ultimately resulting in balanced and largely positive media coverage.

The combination of targeted messaging, community engagement, and strong media relationships contributed to increased public confidence in the program. Additionally, the experience highlighted the importance of flexibility, preparedness, and the ability to respond quickly in a dynamic media environment. These efforts not only supported the successful implementation of the

pilot program but also strengthened the District's reputation as a trusted public health agency committed to transparency and innovation.

Conclusion

The SIT pilot program demonstrated that effective communication and public outreach are essential components of implementing innovative vector control strategies. A proactive, transparent, and community-focused approach can significantly enhance public understanding and acceptance, even when introducing new and complex technologies.

The lessons learned from this pilot will inform future outreach efforts as the District continues to explore and expand innovative mosquito control strategies. By prioritizing clear communication, strong partnerships, and community engagement, public health agencies can successfully navigate challenges and build lasting trust with the communities they serve.

Acknowledgements

The Sacramento-Yolo Mosquito and Vector Control District staff for their collaboration and dedication in supporting both the operational and outreach components of the SIT pilot program.

Thirty years as a ‘pest detective’: Seasonal and long-term trends

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Introduction

The Contra Costa Mosquito and Vector Control District (District) provides a courtesy pest identification service for County residents. For the past 23 years, electronic records of most of these requests have been retained and categorized by type of pest, revealing both seasonal and long-term trends.

Methods

Although the District website specifies that, as a public health agency, we accept ‘biting or stinging pests only’, a wide variety of requests have been received and samples rarely are refused once submitted. We do not accept submission of large items like furniture or bags of clothing which would be time-consuming to examine, or samples of a medical nature like skin scrapings and bodily fluids. We do not offer diagnosis of insect bites, skin lesions or other potential medical conditions. Samples may be dropped off at our office during business hours or mailed to us in secure containers or sealable plastic bags. We also occasionally receive photographs of pests via email, and do our best to identify them, although the resolution may be insufficient for accurate identification, and actual specimens are preferred. Laboratory staff usually respond to these requests by the following business day, via phone or email, and for the past 23 years, the results have been categorized and recorded in a database, which enables us to examine seasonal and long-term trends.

Results and Discussion

The most common category of submissions was ticks (Table 1). We provide identification, information on potential disease risks, and referrals to commercial and non-commercial laboratories that provide testing for *Borrelia burgdorferi* and other tickborne pathogens. Tick submissions show clear seasonality, consistent with the biology of the respective species. For example, *Ixodes pacificus* adults are most commonly submitted during the cooler months of the year, and *Dermacentor* species during the warmer months (Fig. 1). The next largest category was mosquitoes. We encourage members of the public to provide samples when they request service, and our field technicians

Table 1.—Pest samples submitted by category, 2002–2025.

Tick	1,232
Mosquito	354
Yellowjacket	268
Other insect	202
Other	199
No insect/vector	149
Mite	133
Bee	87
Spider	77
Gnat/Midge	53
Fly	52
Pantry pest	46
Termite	35
Flea	27
Unknown	23
Ant	22
Wasp	19
Cockroach	12
Rodent	10
Bed Bug	2
Head lice	2
Other vertebrate	2

also sometimes submit samples they collect on-site while responding to service requests. As with ticks, mosquito submissions clearly reflect the seasonal activity patterns of each species, with univoltine species such as *Aedes washinoi* and *Aedes sierrensis* reported primarily in spring (Fig. 2a),

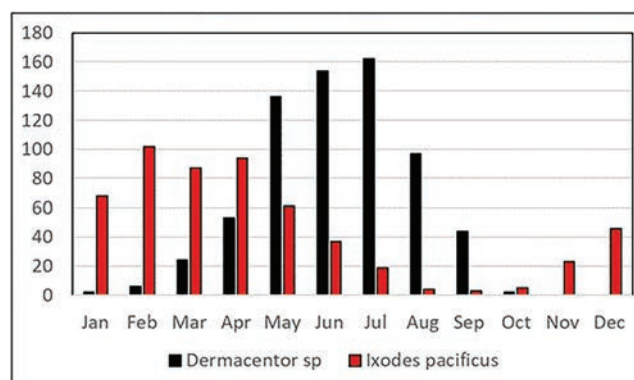


Figure 1.—Tick samples identified, 2002–2025, by month and species.

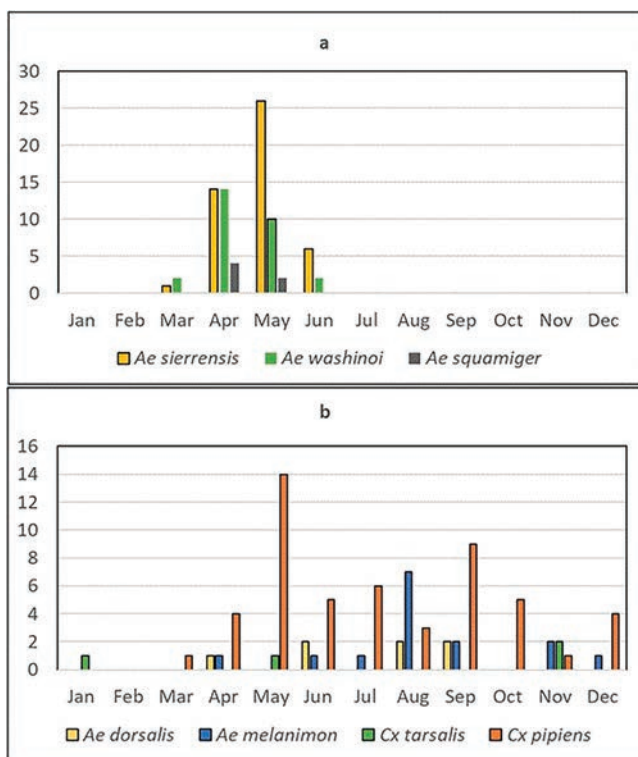


Figure 2.—a. Univoltine mosquito samples identified, by month and species. b. Multivoltine mosquito samples identified, by month and species (multivoltine species).

and multivoltine species such as *Culex tarsalis* reported year-round (Fig. 2b).

‘Other insect’ refers to a variety of household and garden pests. We will identify the specimens submitted and provide information about potential health or nuisance issues by providing links to other, more detailed sources of information including links to detailed brochures provided

by the University of California Integrated Pest Management Program (<https://ipm.ucanr.edu/PMG/menu.homegarden.html>). Plant or garden pest questions also may be referred to our County Department of Agriculture or UC Cooperative Extension for more detailed information. We encourage the caller to try to identify the source or point of entry of the pest, and may recommend they contact a licensed pest control company if they are not comfortable dealing with the issue themselves. We do not recommend specific pest control companies or pesticide products, and encourage callers to always follow label instructions if they choose to apply pest control products on their own.

‘No insect/pest found’ refers to cases of delusional parasitosis, also known as Morgellons disease (Pearson et al, 2012), where the caller reports experiencing ‘bites’ or other symptoms and may submit multiple samples, usually consisting of lint and other random household debris, but no actual pests present. Because this is a medical diagnosis we are not legally qualified to make, we inform the caller that no pest was found, and may recommend that they consult a dermatologist who would be better equipped to make a diagnosis. At least one dermatology clinic we have worked with refers samples submitted by patients to us, and we provide a written report to the doctor rather than dealing with the patient directly. This is the most appropriate way to handle such cases, and is a good example of the One Health concept (One Health Commission, 2026).

‘Other’ refers to biological and non-biological samples that are not vector or insect-related but have been mistaken for or suspected to be pests, and have included snail eggs, plant seeds and parts, lint, fibers and fungi.

Over the past 23 years the numbers of pest identification requests have decreased (Fig. 3). This may be due to increased reliance by the public on online resources for pest identification and information rather than relying on local public agencies. This reduces the workload on our staff, but also increases the chance for misinformation if the

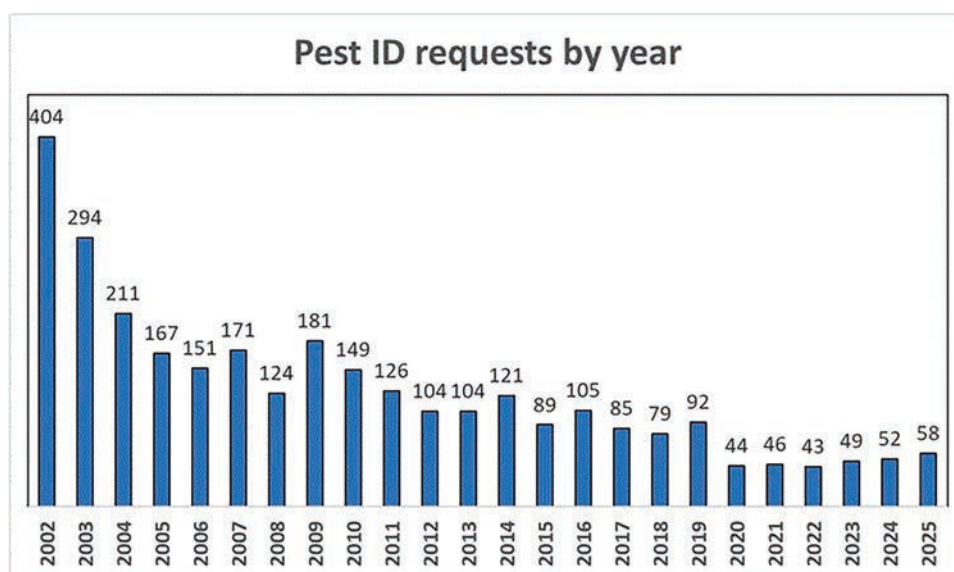


Figure 3.—Pest identification requests by year, 2002–2025.

information sources are not verified or the identification is incorrect.

Some of the partnerships with other agencies and entities have been extremely helpful in providing pest information to the public. These have included the University of California Statewide IPM Program, which provides an outstanding set of online brochures on a wide variety of household, garden and agricultural pests, the Contra Costa County Department of Agriculture, and the California Department of Public Health Vector-Borne Disease Section. We have also worked with local veterinarians, a local dermatology group, and a former Pets and Wildlife columnist for our local newspaper, who has referred many photos and samples from her readers to us, and occasionally published our responses in the newspaper, in addition to helping us keep the public informed about our vector control and disease surveillance programs. These partnerships are a good illustration of the

One Health concept (One Health Commission, 2026) in practice.

Acknowledgements

The author wishes to thank the Laboratory, Public Affairs and Office staff at the Contra Costa Mosquito and Vector Control District for their assistance in dealing with pest identification requests and interacting with the public.

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From EPA/CDPR Greenlight to real world deployment: MosquitoMate's 2025 California rollout — and beyond

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Abstract

Wolbachia-based Incompatible Insect Technique (IIT) offers a non-insecticidal, species-specific approach to suppressing *Aedes aegypti* and *Aedes albopictus*. By releasing male mosquitoes that carry *Wolbachia* and cannot produce viable offspring when mating with wild females, IIT can reduce mosquito populations and complement integrated mosquito management. This presentation traces MosquitoMate's progression from regulatory milestones to operational deployment, focusing on the 2025 rollout in California and the roadmap for further expansion. Practical elements were outlined for adding IIT to a typical abatement program: for those simply wanting additional control tools and for those wanting to extend prior scientific studies. For example, what are the logistics of a typical week in which IIT males are released? Can IIT be overlayed onto existing conventional control and monitoring? This presentation highlighted recent work with multiple California Mosquito Control Districts, sharing lessons from prior operational activities. The presentation addressed the question: For 2026, how can *Wolbachia* IIT fit into existing control portfolios to reduce *Aedes*-borne risk while maintaining public acceptance?

A pilot application of *Wolbachia*-infected males (WB1) to suppress *Aedes aegypti* populations in San Joaquin County

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Introduction

Aedes aegypti was first detected in San Joaquin County in 2019. Since its initial discovery, this species has demonstrated a rapid and aggressive expansion across the region. By 2024, this mosquito had successfully reached all incorporated cities within the county (Figure 1). Many of these areas are now widely infested, with some urban areas experiencing high population densities that have significantly overwhelmed the San Joaquin County Mosquito and Vector Control District (District) capacity for service requests and traditional control demands. For example, the number of service requests for mosquito problems in 2024 in the City of Manteca has increased tenfold compared to 2023. This widespread distribution and the resilient nature of the species underscore an urgent need for innovative mosquito control technologies to enhance Integrated Vector Management (IVM) of *Ae. aegypti*.

One such innovation is the Sterile Insect Technique (SIT) that has been evaluated for many years under the EPA's experimental permit (Crawford et al. 2020). In 2025, MosquitoMate WB1, an SIT product based on *Wolbachia*-infected males, was registered in California for operational mosquito control. This technology relies on *Wolbachia*-infected Cytoplasmic Incompatibility (CI), in which mating between *Wolbachia*-infected males and *Wolbachia*-free wild females results in non-viable eggs, leading to a decline in target wild populations over time. In 2025, the District conducted a pilot application of WB1 in the City of Ripon, California, to evaluate its efficacy in suppressing wild *Ae. aegypti* populations. The primary goal was to compare the efficacy and cost-practicality of WB1 SIT against a combination of conventional methods, including larvicide applications, ground adulticide ULVs, and areawide source reduction.

Method

Study Design and Site Selection. The pilot study was conducted in north Ripon, CA, where *Ae. aegypti* populations were present in moderate to high levels (Figure 2). Two distinct zones of residential areas were used to compare WB1 application and conventional IVM methods (Figure 2). A 37 acre SIT zone was designated for the release of MosquitoMate WB1 males only, and no other

control measures were used. Within the WB1 release period, the 42 acre non-SIT zone was treated with three ULV applications of the adulticide DeltaGuard (Deltamethrin), one WALS application of Altosid Liquid larvicides, and area-wide source reduction.

WB1 Release Rate. In the SIT zone, releases were conducted at the EPA-approved maximum rate of 1,200 males per acre per week. WB1 males were scheduled to be delivered to the District's lab on Thursday mornings and were then immediately transported to the field for release. The release program spanned a 16-week period, starting from 12 June and concluding on 2 October, 2025. The scheduled release at week 5 was skipped due to shipping issues that resulted in 100% mortality of the to-be-released WB1 males.

Surveillance and Statistical Analysis. Mosquito populations in both zones were monitored using BG-sentinel traps baited with CO₂. Traps were deployed at fifth day post-release and collected after 24 hours (six days post-release). Trap locations were shown in Figure 2. For the purpose of data analysis, only female counts were utilized to measure the impact on the wild population. A negative binomial generalized estimating equations (GEE) regression model was used to estimate weekly population growth rates. This model accounts for longitudinal, clustered and overdispersed mosquito surveillance data.

Result and Discussion

The initial population levels in each zone were not significantly different and fluctuated in parallel during the first eight weeks (Figure 3). From week 9 through the final release, population levels differed significantly, with the WB1 application achieving consistently better population control compared to conventional methods.

Statistical modeling revealed that the population growth rate in the SIT zone was 5.7% per week, which was significantly lower than the 10.8% weekly growth rate observed in the non-SIT zone ($\beta = -0.026$, $P = 0.018$)(Figure 4). During the release period, the average female count per trap was approximately 14.5 females per trap-day in the non-SIT zone and 10.0 in the SIT zone. However, trap counts at these levels are sufficient to trigger service requests. It has been reported that multiple releases per week may be required to achieve greater population

The first detection of *Aedes aegypti* in each location by Year, 2019-2025

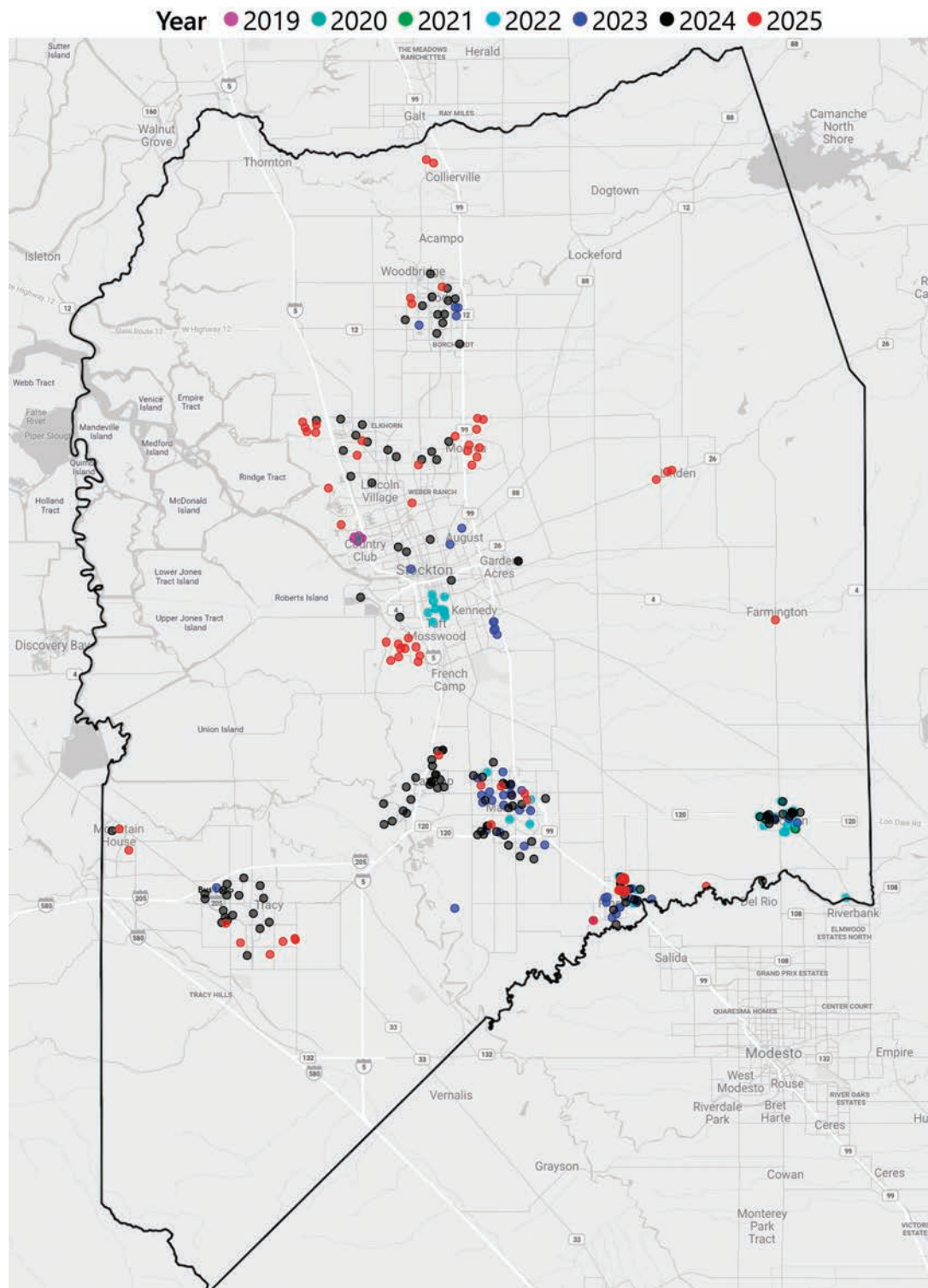


Figure 1.—First detection of *Aedes aegypti* in each location by year (2019–2025).

suppression (Bansal et al. 2024; Crawford et al. 2020). We conducted one release per week due to constraints in time and labor resources. This may have resulted in an insufficient number of sterile males to effectively compete with wild males

during the latter part of the release week. Future strategies might benefit from splitting releases into two events per week (e.g., 600 males per acre twice weekly recommended by the manufacturer) or even more releases per week.



Figure 2.—Map of the application zones and BG2 trapping locations.

The pilot application of WB1 demonstrated that although the sterile insect technique (SIT) showed greater biological efficacy, it posed significant challenges in terms of resource allocation. A comparative cost analysis for this pilot project highlighted substantial differences between the two approaches. Yearly material costs for SIT were estimated at approximately \$1,000 per acre, compared to \$40 per acre for conventional control methods. In addition, the SIT program required approximately 150 staff hours, whereas the conventional control zone required fewer than 50 staff hours.

In conclusion, MosquitoMate WB1 is a powerful tool for suppressing *Ae. aegypti* populations, but the logistical challenges and significantly higher material and labor costs represent a major barrier to wide-area implementation. SIT may currently be most practical as a targeted “hotspot” suppression tool rather than a county-wide replacement for traditional IVM.

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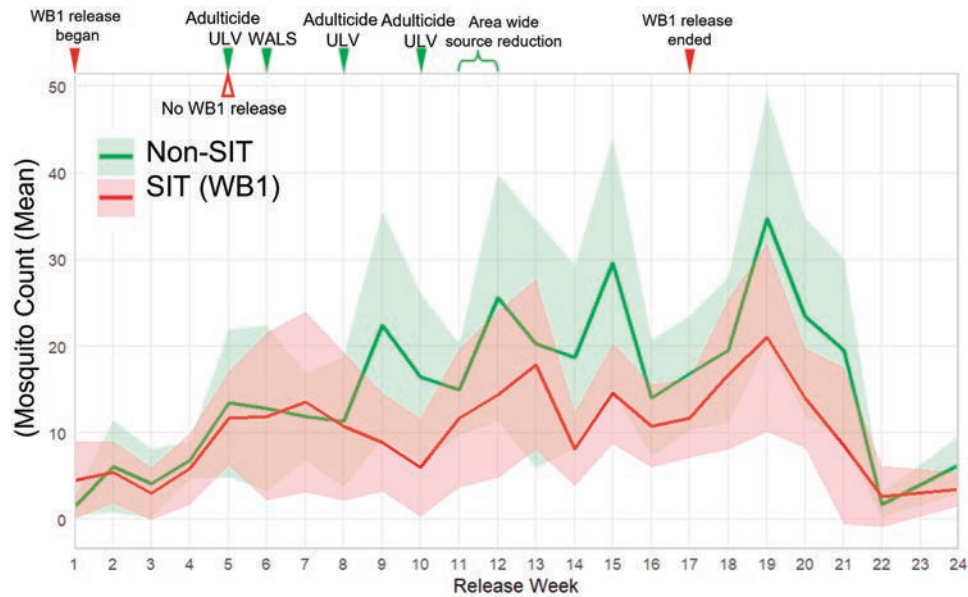


Figure 3.—Observed *Aedes aegypti* population trends in non-SIT and SIT zones. Shaded areas represent the standard deviation of observed trap counts. Control and release events during the pilot application period are depicted at the top of the chart. Red triangles indicate events occurring in the SIT zone; green triangles indicate events occurring in the non-SIT zone.

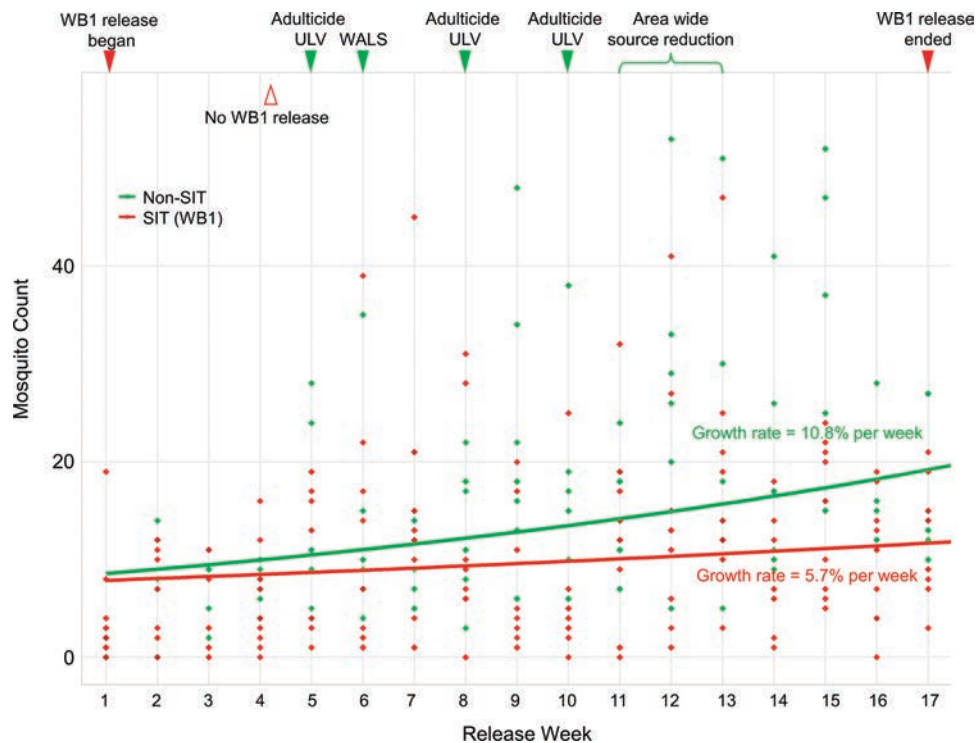


Figure 4.—Projected *Aedes aegypti* population trends in non-SIT and SIT zones. Red dots represent observed female counts in each BG-Sentinel trap in the SIT zone; green dots represent observed female counts in each BG-Sentinel trap in the non-SIT zone. Control and release events during the pilot application period are depicted at the top of the chart. Red triangles indicate events occurring in the SIT zone, and green triangles indicate events occurring in the non-SIT zone.

Trial releases of *Wolbachia* infected male *Aedes aegypti* as part of an integrated mosquito management program

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Introduction

The invasive mosquito *Aedes aegypti* has expanded its distribution and abundance in Sacramento and Yolo Counties since 2019, increasing the need for effective control strategies in urban environments. Wide Area Larvicide Spraying with VectoBac WDG (WALS) is routinely used to target cryptic larval habitats, but additional tools are needed to further suppress adult populations. Releases of *Wolbachia* infected male mosquitoes (WB1; MosquitoMate) provide a complementary approach by reducing egg viability through cytoplasmic incompatibility. In 2025, the Sacramento Yolo Mosquito and Vector Control District evaluated the integration of this sterile insect technique with WALS for the control of *Ae. aegypti*.

Methods

A 25-acre study area in South Natomas, Sacramento County, CA, was selected for a pilot release program. *Wolbachia* infected male *Ae. aegypti* were released twice weekly from 8 Jul through 23 Oct 2025 at a rate of 600 males per acre per release or 1,200 males per acre per week. WALS applications of VectoBac WDG were conducted in three cycles using a three week on and three week off schedule with the first application on 12 Jun and the last on 17 Sep 2025.

Four treatment zones were established including SIT plus WALS, buffer with WALS (200 m zone surrounding the release area), WALS only, and untreated control. Mosquito abundance was monitored weekly using BG Sentinel traps with fifteen traps per zone. BG traps were set with dry ice and BG lure. Male mosquitoes collected in traps were tested for *Wolbachia* infection using a quantitative polymerase chain reaction to estimate the dispersal of WB1 males and the ratio of WB1 males to wild type males, with the goal of 10 WB1: 1 wild type.

Female *Ae. aegypti* abundance measured as trap counts was analyzed using a zero inflated negative binomial mixed model to account for overdispersed count data with excess zeros, with traps included as a random effect. *Wolbachia* detection in male mosquitoes was evaluated as presence or absence using a binomial generalized linear mixed effects model with distance from the release boundary as a fixed effect and site as a random intercept.

Results and Discussion

Releases achieved consistent overflooding within the treatment area, with a mean ratio of 11.6 to 1 *Wolbachia* positive to wild type males. Ratios declined rapidly outside the release area, indicating limited dispersal beyond the treatment zone. In agreement, detection of *Wolbachia* infected males declined sharply with distance, with an estimated 71% reduction in the odds of detection per 100 m from the release area boundary. This steep spatial gradient indicated that the operational impact of sterile male releases was largely confined to the release area.

WALS applications alone significantly reduced female *Ae. aegypti* abundance, with mean reductions of 46.8% relative to the control. The addition of sterile insect technique provided further suppression, with the SIT plus WALS treatment resulting in approximately 25 to 40% further decrease in female abundance than WALS alone during weeks 10 through 19. This difference increased over time, indicating the cumulative effect of sterile male releases.

Despite detectable movement of WB1 males into the buffer zone, no measurable reduction in female abundance was observed outside the release area, suggesting that suppression requires sustained overflooding ratios within the target zone.

Conclusions

WALS produced substantial reductions in *Ae. aegypti* abundance, and the addition of *Wolbachia* based sterile insect technique provided further suppression within the release area. However, the effect of sterile male releases was spatially constrained, emphasizing the importance of achieving adequate coverage and overflooding across the treatment area. Integrated control strategies that combine larval and adult interventions show promise, but require continued refinement to maximize effectiveness.

Acknowledgements

We thank the staff of the Sacramento Yolo Mosquito and Vector Control District for field and laboratory support, the residents of South Natomas for their cooperation.

Laying the groundwork for SIT releases: *Wolbachia* trials in the San Gabriel Valley

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Abstract

The San Gabriel Valley Mosquito and Vector Control District (District) has spent the past year establishing the operational, technical, and community foundations necessary for future Sterile Insect Technique (SIT) releases using *Aedes aegypti* males infected with *Wolbachia*, produced by MosquitoMate, Inc. As vector control programs explore emerging biological tools, early preparation and rigorous local testing are essential to ensure effectiveness, safety, and public understanding. The District's phased approach emphasizes both scientific validation and transparent communication to support long-term integration of SIT into regional mosquito management. Initial in-house health and fitness evaluations were conducted to assess the performance and competitiveness of the MosquitoMate strain under local environmental conditions. Concurrently, the District launched Mosquito Match, a public-facing outreach program designed to explain the science behind SIT, the mechanisms of *Wolbachia*-induced incompatibility, and the District's preparatory work. In late 2025, a Mark-Release-Recapture (MRR) study was completed to evaluate dispersal and survival of released males, with a second MRR planned for early summer 2026. These trials were designed to refine field parameters and logistics in advance of a 16-week operational release and evaluation phase. Through this systematic, data-driven process, the District is laying the groundwork for future SIT implementation, ensuring readiness to incorporate *Wolbachia*-based suppression into modern integrated vector management practices.

Operational evaluation of *Wolbachia*-Infected male *Aedes aegypti* releases for population suppression in Parlier, California

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Introduction

The control of invasive *Aedes aegypti* remains a significant operational challenge for mosquito and vector control districts in California due to its use of container larval habitats and close association with human environments. *Wolbachia*-based sterile insect technique (SIT) approaches have demonstrated efficacy in prior studies conducted under Experimental Use Permits (EUP) and Research Authorizations (RA). More recently, *Wolbachia*-infected male mosquitoes have become commercially available for operational use; however, tube-based delivery and release programs have only been evaluated under EUP and RA conditions in California.

Our study evaluated the effectiveness and operational feasibility of releasing *Wolbachia* (wAlbB strain WB1)-infected male *Ae. aegypti* at label rates to suppress a wild population under routine operational conditions in Parlier, California.

Methods

A 25-acre treatment area in Parlier, California, received releases of *Wolbachia*-infected male *Ae. aegypti*, with Kingsburg, California, serving as a non-release control. Approximately 30,000 males were released weekly across 25 evenly distributed point locations (Fig. 1) during two release events per week.

Mosquitoes were retrieved upon arrival at a shipping facility in Clovis, California, and transported to the District laboratory in Parlier. Tubes were transferred from shipping containers to insulated tote bags for field deployment. Releases were conducted under mild weather conditions (52–95°F). When temperatures exceeded acceptable thresholds, mosquitoes were held overnight and released the following day. Releases were conducted by 2–4 staff and completed in approximately 20 minutes.

Male mosquitoes that were dead or unable to fly were excluded from release. Non-released individuals were returned to the laboratory and categorized as dead or moribund to estimate release efficiency.

Adult mosquito abundance was monitored weekly using BG-Sentinel 2 traps baited with BG-Lure and dry ice. Twelve trap sites were established in and around the treatment area, including seven within or along the perimeter. Twelve additional sites were monitored in the control area. Suppression was calculated as the percent reduction in female abundance relative to the control site, excluding perimeter traps.

Results and Discussion

Release operations were conducted efficiently, with an average non-release rate of approximately 8%. From weeks 33 to 40, suppression in the treatment area ranged from 21% to 82% (Figure 2), with peak suppression observed in week 40. Female *Aedes aegypti* abundance in Parlier remained consistently lower than in the control area for approximately six weeks following initial releases, although suppression levels varied over time.

Interpretation of results should consider confounding factors, including rainfall (week 38 excluded), two wide-area larvicide applications during weeks 38 and 39, and natural seasonal population declines. Despite these factors, consistent reductions relative to the control site indicate that *Wolbachia*-based releases contributed to population suppression under operational conditions.

Conclusions

Releases of *Wolbachia*-infected male *Ae. aegypti* resulted in measurable population suppression and demonstrated operational feasibility; however, suppression levels were not consistently sustained throughout the release period. Previous evaluations of releases by the District indicate greater effectiveness when releases are initiated prior to seasonal population increases, whereas the current study began releases in mid-June.

Although average mortality within release tubes was less than 10%, no assessment of male competitiveness or overflooding ratios were conducted. As a result, the



Figure 1.—Treatment area (~25 acres) in Parlier, California, showing the distribution of 25 release point locations (orange) and 12 BG-Sentinel trap locations (blue) used for weekly adult surveillance.

effective release ratio relative to the wild population remained uncertain. Ensuring male fitness and achieving sufficient overflooding ratios are critical factors that warrant further evaluation.

This IIT approach showed promise as a complementary tool within integrated vector management programs targeting invasive *Ae. aegypti*. Future operational use should consider earlier release

timing and improved methods for assessing male quality and sufficient release rates.

Acknowledgements

The authors thank District staff Katherine Brisco, Connor D’Souza, Melissa Theis, and Clarita Ramblas for their operational support and field implementation.

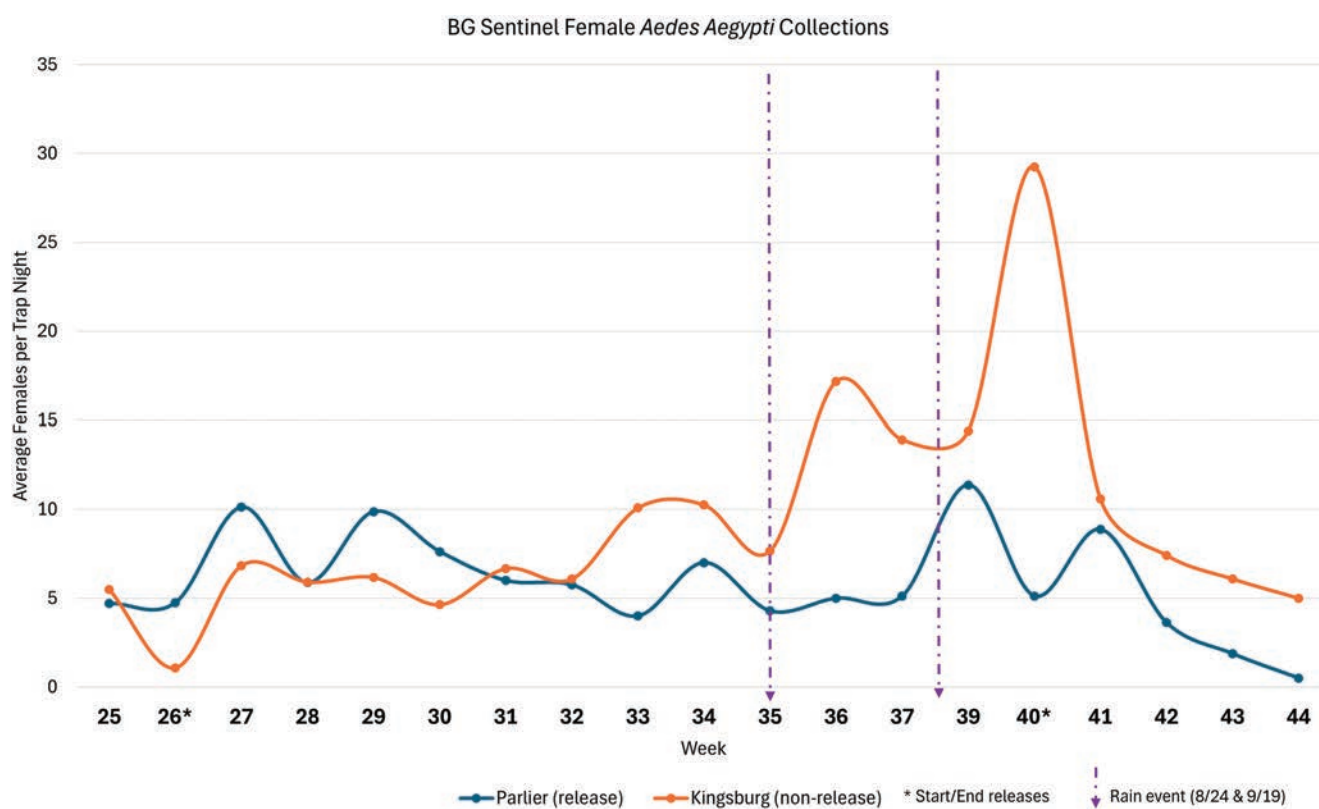


Figure 2.—Weekly average female *Aedes aegypti* abundance per BG-Sentinel trap night in the Parlier treatment (release) and Kingsburg control (non-release) areas, weeks 25–44. Vertical dashed lines indicate rainfall events, and asterisks mark the initiation and conclusion of releases.

Advancing *Aedes* control: Results from year two of the SIT program in the West Valley region in Southern California

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Introduction

Invasive *Aedes aegypti* mosquitoes continue to expand across California, increasing the risk of locally transmitted arboviruses, including dengue. Traditional control strategies alone often become insufficient because of the cryptic and highly urbanized larval habitats utilized by invasive *Aedes* mosquitoes. Consequently, innovative tools are required to strengthen the existing integrated vector management (IVM) programs. The Sterile Insect Technique (SIT), which involves releasing sterilized male mosquitoes to mate with wild females, resulting in unviable eggs that lead to suppression of the mosquito population, has emerged as a promising complementary control strategy. This study summarized results from the second year of an operational SIT program implemented by the West Valley Mosquito and Vector Control District (WVMVCD) in Southern California targeting invasive *Ae. aegypti* populations.

Methods

The WVMVCD SIT program was developed through a multi-year process involving feasibility studies, regulatory approvals, colony establishment, irradiation optimization, survivorship and fitness experiments, and extensive public outreach. Historically identified *Aedes* hotspots were selected for operational SIT releases, with 31 sites included during 2024 and 2025. Less than 48-hour-old male *Ae. aegypti* were sterilized using X-ray irradiation and released biweekly between April and November. Continuous weekly monitoring was conducted using BG-Sentinel traps to evaluate mosquito abundance and spatial trends. Mosquito population changes were assessed using female *Aedes* mosquitoes per trap-night (Ae/TN), and analyses examined treatment responses across varying mosquito density categories and wild-to-sterile release ratios. Service requests related to *Aedes* mosquitoes also were evaluated as an indicator of operational impact and mosquito nuisance reduction.

Results

Aedes populations consistently declined over the two years following SIT implementation. Mean female

Ae. aegypti trap-night counts declined from 18.6 Ae/TN in 2023 to 13.5 in 2024 and further to 10.5 in 2025. Suppression effects were observed within approximately 150 m of sterile male release sites. High-density sites (> 50 Ae/TN) demonstrated the strongest response, with mosquito populations declining by approximately 82% between 2023 and 2025. Medium-density sites (20–50 Ae/TN) declined by 66%, while low-density sites (< 20 Ae/TN) declined by 57%. Significant reductions were also observed across varying wild-to-sterile release ratios. Even moderate release ratios (30–49X) resulted in approximately 25% population reduction, while release ratios exceeding 100X produced reductions approaching 48%. *Aedes*-related resident service requests declined by approximately 34% in 2025 compared with 2024, suggesting reduced mosquito nuisance complaints and possible public relief. District-wide spatiotemporal trends further demonstrated reduced mosquito densities, with nearly 80% of all monitored traps recording fewer than 20 Ae/TN in 2025. Operationally, the in-house X-ray-based SIT program substantially increased program capacity while reducing staffing and material costs compared with outsourced approaches.

Conclusion

The results demonstrated that integrating SIT into existing IVM programs can substantially reduce invasive *Ae. aegypti* mosquito populations in urban Southern California environments. Suppression effects were observed across varying mosquito densities, including historically high-density hotspots. Notably, even lower wild-to-sterile ratios resulted in measurable *Aedes* mosquito population reductions. In addition to entomological suppression, reductions in *Aedes*-related service requests suggest operational and community-level benefits. These findings support the value of operational SIT programs as sustainable and scalable complementary tools for invasive *Aedes* control. Continued optimization of release strategies, density-dependent suppression analyses, and integration with other IVM interventions may further enhance long-term mosquito suppression and reduce arboviral transmission risk.

From Lab to Landscape: Year two progress of sterile male releases in Los Angeles County

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Introduction

The establishment of invasive *Aedes aegypti* in Southern California since their initial detection in 2014 presents a severe and escalating challenge to public health agencies in the region (Metzger et al. 2015). *Aedes aegypti* is the primary vector of viruses causing dengue, chikungunya, Zika and yellow fever (Souza-Neto et al. 2019). Historically, symptomatic cases within the state were exclusively associated with travel exposure. However, in 2023, the first locally transmitted cases of dengue were identified in Los Angeles County, with further cases identified in 2024 and 2025 (CDPH 2025). This emerging threat of local dengue virus transmission necessitates an immediate reevaluation of regional vector management protocols.

Controlling *Ae. aegypti* has proven difficult using traditional mosquito control strategies. The species is highly adapted to human environments, utilizing cryptic, often inaccessible urban breeding sites that are difficult to treat with standard larvicides. Furthermore, the efficacy of adulticiding efforts is increasingly compromised by widespread and growing pyrethroid insecticide resistance among California populations (Yang et al. 2020).

Given these operational limitations, the Sterile Insect Technique (SIT) offers a species-specific, environmentally sustainable alternative. SIT is an area-wide method of biological control that involves the mass-rearing, sterilization, and systematic release of male insects to mate with wild females, thereby reducing the reproductive potential of the target population (Alphey et al. 2010; Bourtzis and Vreysen 2021). Because released sterile males actively seek out wild females, SIT effectively bypasses the logistical challenge of locating cryptic urban larval sites.

Although agricultural SIT programs have a long history of success, the application of X-ray sterilized *Ae. aegypti* SIT within dense, topographically complex urban landscapes represents a new frontier (FAO/IAEA 2020). Programs abroad, as well as recent pilot studies in the United States, have demonstrated promising results in suppressing localized *Ae. aegypti* populations (Morreale et al. 2025). To evaluate the operational

feasibility and density-dependent thresholds of this strategy in Southern California, the Greater Los Angeles County Vector Control District (District) launched an *Ae. aegypti* SIT pilot program, beginning with baseline surveillance in 2022 and initiating active releases in 2024. The current study examined the efficacy of this pilot program in a semi-urban environment, assessing its impact on wild female abundance, induced sterility rates, and community perception over a two-year period.

Methods

Study Area and Rearing

The study was conducted in the Sunland-Tujunga neighborhood of Los Angeles County, California (Figure 1). The site comprised three designated 50-acre areas: an untreated control area and two intervention areas (Alpine and Renaissance). These areas support established *Ae. aegypti* populations and are primarily bounded by natural hills, which restrict *Ae. aegypti* dispersal (Figure 2).

To establish a colony of a local strain, *Ae. aegypti* eggs collected from various sites throughout the District were sent to MosquitoMate, Inc. (Louisville, KY) for colony establishment. MosquitoMate subsequently produced and shipped predetermined quantities of eggs (approximately 6,500 eggs/capsule) monthly to the District. In Year 1 (2024), mass-rearing was conducted in a small insectary at District headquarters. In Year 2 (2025), to support larger-scale releases, operations were transitioned to a custom modular insectary constructed within a retrofitted shipping container.

Eggs were flooded (one capsule per pan) and reared *en masse* following standard MosquitoMate protocols at a maintained water temperature of $25.0 \pm 0.5^\circ\text{C}$ (Dobson et al. 2023). During the pupal stage, males were separated using Fay-Morlan glass-plate separators; females and remaining larvae were discarded. Male pupae were transferred to eclosion containers (24 oz plastic containers filled with 12 oz of aged tap water) housed within 12 × 12 in. BugDorm cages (Forestry Suppliers, Jackson, MS) and provided a 10% (w/v) sucrose solution *ad libitum*.

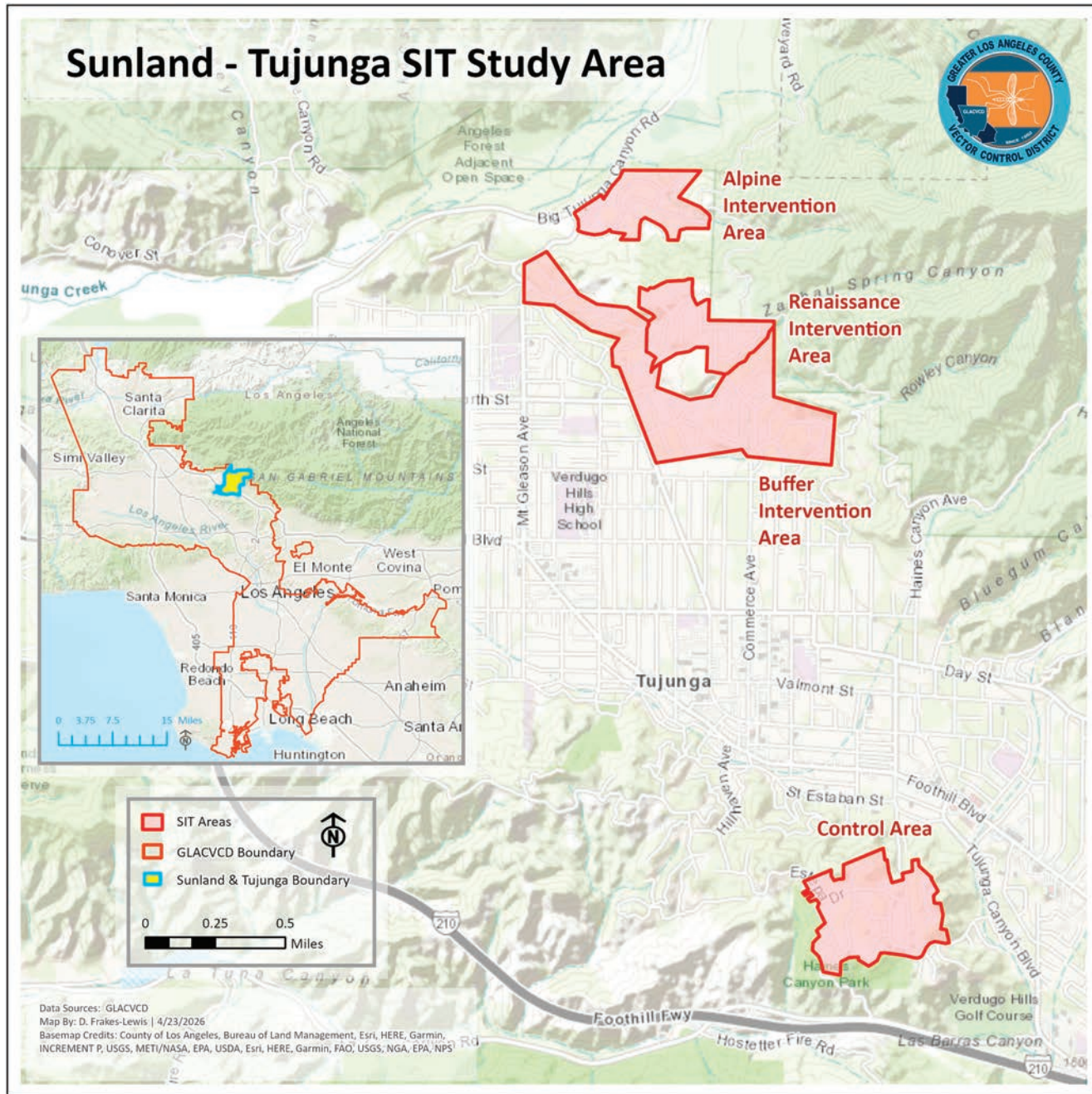


Figure 1.—Map of SIT study areas outlined in red in the Sunland-Tujunga neighborhood in the Crescenta Valley, Los Angeles, California with inset showing GLACVCD boundaries within the County of Los Angeles.

Irradiation

Two days post-sorting, eclosion containers and remaining pupae were removed. Cages were transported to the Orange County Mosquito and Vector Control District (Garden Grove, CA) and placed in a 4°C cold room for 15 minutes. To prevent physical damage during immobilization and to maintain a consistent dosimetric profile, chilled males were compacted to a density of 100 mosquitoes/mL (Chung et al. 2018) before receiving a sterilizing 70 Gy X-ray dose via an RS-1800 irradiator (Rad Source Technologies, Buford, GA).

Post-irradiation, males were re-chilled at 4°C, transferred to 2.5 qt cylindrical plastic release containers, and marked via insufflator with fluorescent DayGlo Eco powder (Cleveland, OH). Powder colors were rotated weekly for batch identification. Prior to transport, paper towels soaked in a 10% (w/v) sucrose solution were placed over the container mesh to provide hydration and sugar *ad libitum*. In 2024, sterile males were transported and released on the day of irradiation. In 2025, to improve recovery, males were returned to the insectary overnight prior to field release the following day.

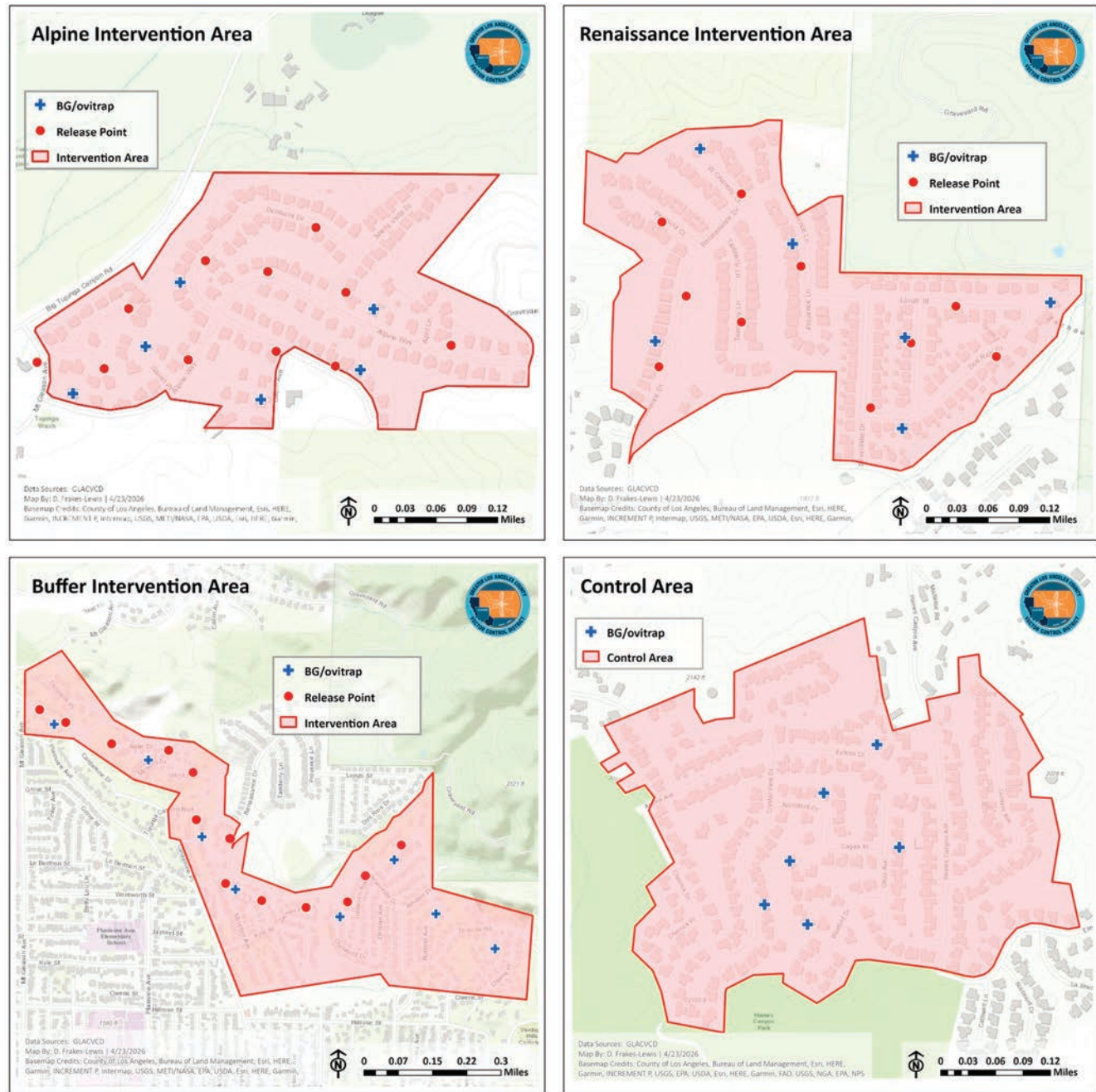


Figure 2.—Map of SIT study areas Alpine intervention area (top left), Renaissance intervention area (top right), Buffer Intervention area (bottom left), and the control area (bottom right). BG/ovitraps trap sites marked with blue crosses and release points marked with red dots.

Intervention Strategy

Surveillance and sterile male releases were conducted from May to October. In Year 1 (2024), Alpine and Renaissance received equal, concentrated releases of sterile males distributed evenly across ten predetermined sites per 50-acre zone. In Year 2 (2025), the per-site release rate within the intervention areas was reduced to evaluate density-dependent suppression thresholds. Additionally, 14 additional release sites were established, including one directly west of the Alpine area as well as 13 release sites in

a buffer zone to the south and west of the Renaissance area to mitigate wild *Ae. aegypti* immigration (Figure 2).

Surveillance and Analysis

Adult abundance and egg hatch rates (sterility) were monitored weekly using a network of 18 trap sites (6 per area). BG-Sentinel 2 traps (Biogents, Regensburg, Germany) baited with BG-Lures and dry ice (CO₂) were deployed overnight, five days post-release, to monitor adult abundance.

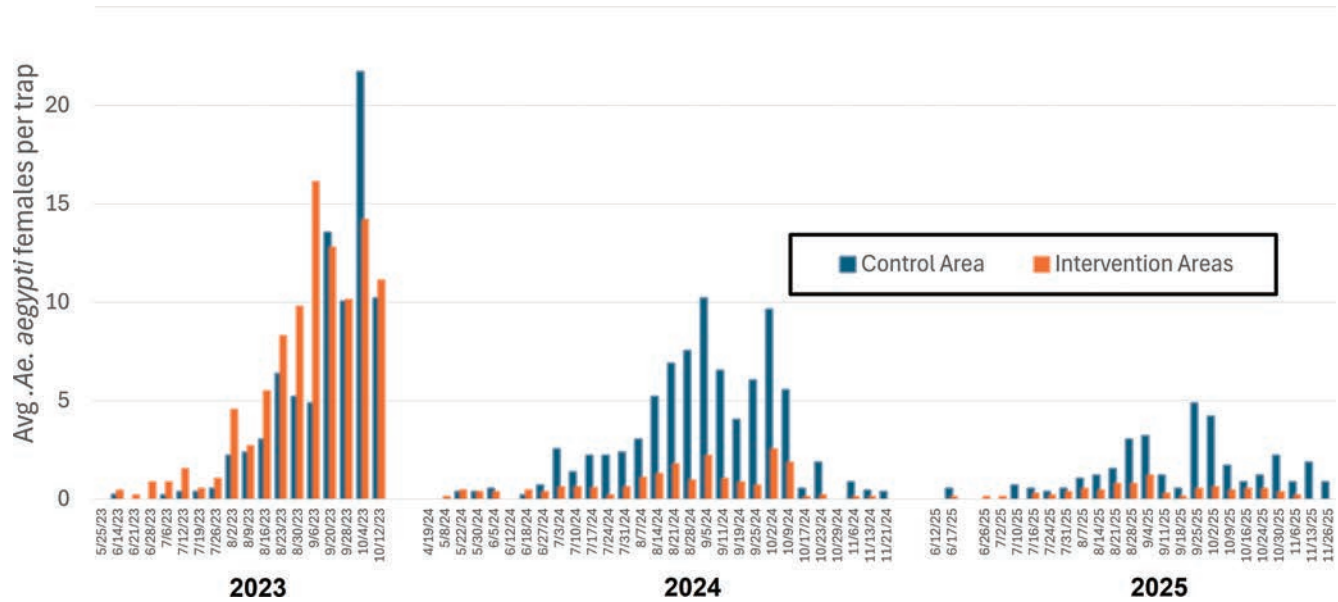


Figure 3.—Average number of adult female *Aedes aegypti* captured per trap per week in the untreated control and sterile male Intervention areas during the 2023 baseline and the 2024–2025 active release seasons.

To monitor oviposition activity, ovitraps were constructed using black one-gallon plastic buckets lined with seed germination paper, filled with tap water, and placed adjacent to each BG trap. Ovitrap papers were collected and replaced weekly. Collected papers were dried and cured for up to three days in a 48 qt cooler acting as a humidity-controlled chamber (maintained at 84% RH via Boveda packs). Following egg counting papers were cut into sections and submerged in 16 oz deli cups containing 300 mL of aged tap and brewer’s yeast. Hatched larvae were counted and recorded after four days to determine the hatch rate.

Statistical analyses were performed in R using the glmmTMB package. To account for overdispersion and a high frequency of zero-counts, female abundance was modeled using a Zero-Inflated Negative Binomial Generalized Linear Mixed Model (GLMM). Trap site and year were incorporated as random effects, and week was modeled as a third-degree orthogonal polynomial fixed effect to account for population seasonality. Sterility was analyzed using a Binomial GLMM comparing total eggs versus viable larvae.

Table 1.—Comparison of female *Aedes aegypti* population reduction in the Alpine and Renaissance intervention zones relative to the untreated Control during Year 1 (2024) and Year 2 (2025) of the SIT pilot program.

Phase	Area	Baseline	Female Reduction	Significance (P)
Year 1 (2024)	Alpine	2024 Control	84.8%	< 0.001
	Renaissance	2024 Control	79.2%	< 0.001
Year 2 (2025)	Alpine	2025 Control	91.4%	< 0.001
	Renaissance	2025 Control	73.5%	< 0.001

Adult population suppression was calculated using Mulla’s formula (Mulla et al. 1971). Wild male *Ae. aegypti* populations in the intervention areas were estimated using the Chapman estimator of the Lincoln-Petersen index to accommodate low recapture rates and occasional zero-counts of sterile males (Chapman 1951).

Community Survey

In October 2023, a knowledge, attitudes, and practices (KAP) survey was conducted in the intervention areas to gauge baseline community knowledge of SIT, satisfaction with District services, and perceived biting pressure. Following two years of releases, a post-intervention survey was repeated in October 2025 in the same neighborhoods to identify longitudinal changes in responses.

Results and Discussion

The implementation of the localized SIT pilot program resulted in clear suppression of wild female *Ae. aegypti* populations during the Year 1 (2024) release period (Figure 3). Although the untreated control area experienced

Table 2.—Total sterile male *Aedes aegypti* released and resulting sterile-to-wild male ratios calculated with recaptures and wild population estimation by intervention area across the two-year SIT pilot program.

Area	Year 1 (2024) # Released	Year 1 Ratio	Year 2 (2025) # Released	Year 2 Ratio
Alpine	276,919	15:1	180,170	17:1
Renaissance	319,777	15:1	164,110	10:1
Buffer	—	—	237,095	—
Total	596,696	—	581,375	—

Table 3.—*Aedes aegypti* egg hatch rates across control and intervention areas during Year 1 (2024) and Year 2 (2025) of the SIT pilot program.

Phase	Area	Hatch Rate
Year 1 (2024)	Control	97.8%
	Alpine	7.9%
	Renaissance	12.8%
Year 2 (2025)	Control	46.8%
	Alpine	0.1%
	Renaissance	44.3%

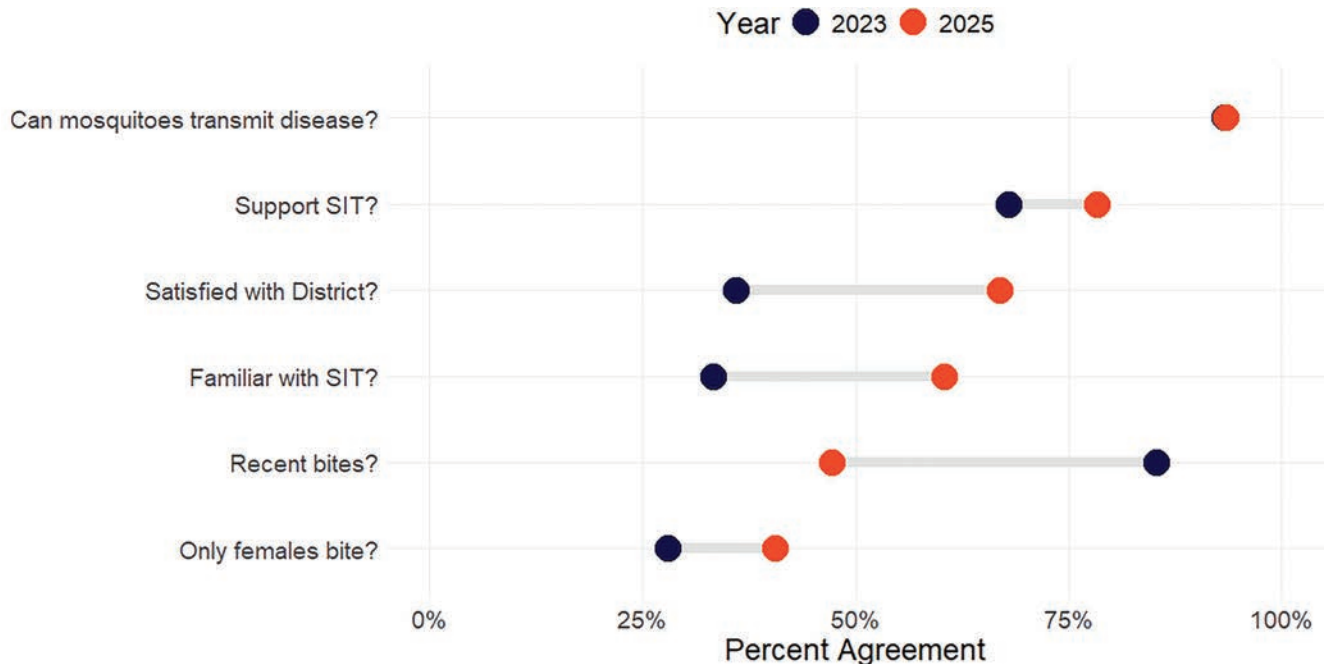
a slight natural decline (12%) in female abundance between 2023 and 2024, GLMM analysis revealed a statistically significant decline in female abundance across both the Alpine and Renaissance intervention areas relative to the control (Table 1). Correspondingly, *Ae. aegypti* eggs collected from ovitraps in both intervention areas exhibited significantly lower hatch rates than those in the control (Table 3). These results demonstrated that weekly releases of irradiated males successfully induced widespread sterility within the wild population, driving the observed reduction in adult female abundance.

During the second season (2025), the total number of sterile males released in the intervention zones was deliberately reduced (Table 2). Despite these lower release numbers, significant suppression of adult female *Ae. aegypti* abundance continued across both areas. However, oviposition surveillance revealed a distinct divergence in sterility metrics: although Alpine

maintained highly suppressed hatch rates, the hatch rate in the Renaissance area rebounded, ultimately mirroring the untreated Control (Table 3). Utilizing the Chapman estimator to model wild male populations, we determined that this loss of induced sterility in Renaissance coincided with a decline in the sterile-to-wild male recapture ratio to below 15:1.

This finding highlights a critical operational threshold for urban SIT. Although adult female abundance may appear suppressed in the short term due to the lagging effects of previous releases, maintaining a strict density-dependent sterile male ratio is paramount to sustaining population suppression and preventing a reproductive rebound. Furthermore, while the addition of the 14-site buffer zone south of Renaissance was operationally feasible, it did not sufficiently mitigate wild *Ae. aegypti* immigration or sustain suppression to the levels observed in Alpine. Additional spatial analysis is required to fully quantify the buffer strategy's limitations.

Beyond the entomological metrics, post-intervention KAP surveys conducted in October 2025 indicated highly positive public reception. Residents within the intervention areas reported a notable decrease in perceived biting pressure and nuisance compared to the 2023 baseline, alongside strong support for the continuation of SIT as a core District service (Figure 4). Ultimately, these results validate the efficacy and public acceptability of urban SIT interventions in Southern California, provided agencies can sustain the rigorous logistical capacity required to meet target sterile-to-wild release ratios throughout the transmission season.

**Figure 4.**—Dumbbell plot illustrating changes in public perception within the SIT intervention neighborhoods from the 2023 baseline to the 2025 post-intervention survey. The connecting lines highlight the shift in the percentage of respondents agreeing with each statement.

Acknowledgements

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Swatting the cost, not the science: Understanding public perceptions of mosquito control and SIT on a budget

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Abstract

The San Gabriel Valley Mosquito and Vector Control District (District), serving nearly two million residents in Los Angeles County, has launched the SGV Mosquito Match program. This initiative involves releasing *Wolbachia*-infected male mosquitoes with the goal of reducing the local *Aedes aegypti* population over time in Bassett, an area with historically high mosquito activity. To assess community perceptions and acceptance of this Sterile Insect Technique (SIT), two surveys were conducted. The first, *SIT Attitudes Survey 2025* (n = 131), gathered general information on mosquito knowledge and perceived mosquito activity levels, familiarity with the District, and opinions about SIT from San Gabriel Valley residents attending community events across the District. The second survey, *SIT in the SGV* (n = 95), specifically targeted residents of La Puente and the unincorporated community of Bassett, where releases were planned. This longer survey measured residents' knowledge of the District, mosquito biology and disease transmission, SIT, and their willingness to support financial investments in the program. Surveys were distributed in both paper and online formats (via SurveyMonkey) through community events, targeted ads, mailers, and door hangers. Traditional door-to-door survey deployment was difficult because of the political landscape and fear of government agencies among the community during the time of distribution. Results indicated residents were concerned about mosquito-borne diseases and value investment in innovative control methods. However, findings also highlight limited awareness of the District and its services, as well as hesitation to support increased financial investment due to personal economic concerns. Vector Control Districts could benefit from similar survey deployments to understand constituent concerns and use the information to create relevant campaigns.

A localized approach to *Aedes aegypti* control: Early findings from a cost-conscious SIT program

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Abstract

The Sterile Insect Technique (SIT) is rapidly becoming an accepted technology for the control of *Aedes aegypti* in the US and globally. In the summer of 2025, Northwest Mosquito and Vector Control District (NWMVCD) implemented a cost-effective SIT pilot program targeting *Ae. aegypti* at Lake Elsinore, California. Local field-collected *Ae. aegypti* eggs were provided to Rad Source Technologies, Inc (hereafter “Rad Source”), a company based in Georgia specializing in X-ray technology and the mass-rearing of insects, to create their *Ae. aegypti* (Cali strain) colony. NWMVCD then purchased the X-ray sterilized male Cali strain mosquitoes from Rad Source, for SIT release. The study site consisted of a 20-acre area in the geographically isolated McVicker Canyon neighborhood of Lake Elsinore with historical *Ae. aegypti* abundance. Within this area we selected three 5-acre treatment zones that received sterile males and one 5-acre control zone that did not receive any sterile males. Routine vector control operations, including pesticide treatments, were performed as usual in all experimental zones. Eight weekly releases of approximately 24,000 sterile males (equivalent to an application rate of 1,600 mosquitoes/acre) were conducted from July to September. Surveillance trapping with BG-Sentinel 2 (n = 13) was conducted weekly, beginning four weeks prior to releases and continuing seven weeks beyond releases to monitor the abundance of *Ae. aegypti*. Prior to field release, adult male *Ae. aegypti* (Cali strain) mosquitoes received from Rad Source were evaluated in-house to assess their longevity and fertility. Preliminary findings point to the operational feasibility of using outsourced sterile males as a cost-effective alternative to in-house rearing and irradiation. Although results did not show strong reduction in the target female *Ae. aegypti* population, NWMVCD demonstrated that SIT can be accessible for vector control agencies without their own sterile male production facility.

Introduction

Aedes aegypti has rapidly expanded in prevalence across Southern California since its initial detection in 2014 (Metzger et al 2015). Control of this species is challenging due to its increasing resistance to insecticides and its strong adaptation to urban environments (Matthews 2019). Additionally, *Ae. aegypti* is a competent vector of dengue, chikungunya, and Zika viruses (Kraemer et al. 2019), with increasing locally acquired dengue cases reported in Southern California since 2024 (California Department of Public Health 2026).

In response to these challenges, a growing number of mosquito control districts throughout California are incorporating Sterile Insect Technique (SIT) into their Integrated Vector Management (IVM) strategies to suppress *Ae. aegypti* populations (Morreale et al. 2025). A primary barrier to implementation is the substantial cost and infrastructure required to establish and maintain a fully in-house SIT program. Successful operation requires technical expertise, sufficiently trained personnel, continuous operations (6-7 days per week), and an appropriately sized insectary capable of housing large mosquito colonies (Foley et al. 2024). These requirements

involve significant upfront and ongoing costs, creating a financial barrier to establishing a program. The Northwest Mosquito and Vector Control District (NWMVCD) evaluated the feasibility of leveraging existing staff, insectary space, and infrastructure, while outsourcing key production components, such as mosquito rearing and irradiation, to develop and implement an SIT program.

Methods

In winter 2024, the NWMVCD collected local *Ae. aegypti* mosquitoes from Corona, CA and reared them to the generation F₁₂. Eggs from the subsequent generation (F₁₃) were sent to Rad Source Technologies, Inc., Buford, GA (hereafter “Rad Source”), a developer of X-ray sterilization systems and large-scale mosquito rearing technologies. Rad Source used the eggs to establish a new *Ae. aegypti* colony, named the “Cali strain”. NWMVCD partnered with Rad Source for mass-production of mosquitoes for implementation of the Sterile Insect Technique (SIT). Rad Source performed all mass-rearing and sex sorting procedures, using a dose of 60 Gray to sterilize the adult males and dusting the mosquitoes using UV-reactive pigment powder prior to shipment. Upon arrival, all mosquitoes were unpacked and held overnight in

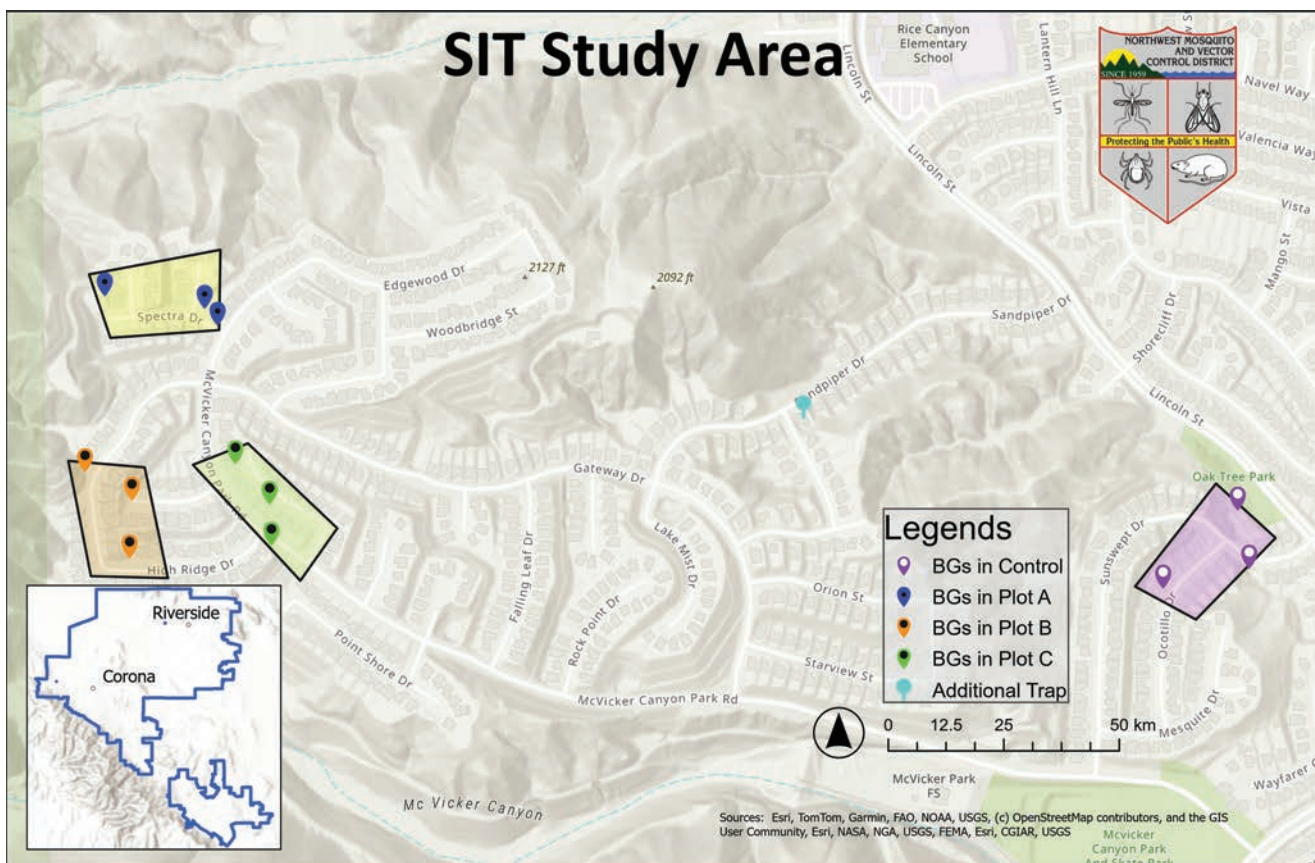


Figure 1.—Map of SIT study area in Lake Elsinore, Riverside, California. Aerial map of district coverage area (bottom left), a magnified aerial view of the study area in McVicker Canyon showing three 5-acre treatment plots A-C (highlighted in blue, orange, and green) and one 5-acre control plot (indicated in purple). Color-coded map pins indicate BG-Sentinel 2 trap locations ($n = 3$ traps per plot). The teal pin depicts an additional BG-Sentinel 2 surveillance trap site.

the NWMVCD insectary under controlled conditions (70-75% humidity, 26°C) prior to release.

Longevity

Prior to field releases of the “Cali strain” *Ae. aegypti*, NWMVCD staff evaluated the median longevity of the irradiated males to determine their potential to survive long enough to mate under field conditions. The initial trials were conducted in winter 2025, comparing non-irradiated laboratory colony *Ae. aegypti* males with “Cali strain” irradiated *Ae. aegypti* males shipped from Rad Source. Mosquitoes were sorted and distributed into cups ($n = 5$ cups per strain, 10 cups total), maintained with 10% sucrose solution under controlled laboratory conditions. Mortality was recorded every 2-3 days. The number of mosquitoes in each cup varied from 4-13 males for the non-irradiated colony (mean = 8.4 mosquitoes) and 10-13 males for the irradiated “Cali strain” (mean = 11.6 mosquitoes). A second trial was performed in summer 2025, concurrent with the SIT releases, and focused exclusively on irradiated “Cali strain” males from Rad Source. Mosquitoes were sorted into cups ($n = 6$ cups) with 10-15 mosquitoes per cup (average = 11.2 mosquitoes) and maintained under the same environmental conditions.

Fecundity

To assess whether the “Cali strain” males of *Ae. aegypti* would mate with laboratory colony females and to verify if the resulting eggs were non-viable, two mating chambers were set up, “Cali” and “Colony”. Chamber “Cali” contained a 1:1 ratio of 10 “Cali strain” *Ae. aegypti* males and 10 laboratory colony *Ae. aegypti* females. Chamber “Colony” contained a 1:1 ratio of 10 laboratory colony *Ae. aegypti* males and 10 laboratory colony *Ae. aegypti* females. Both chambers were maintained under controlled conditions (25°C, 77% relative humidity), provided with 10% sucrose solution. Mosquitoes were allowed to mate for three days before the males were removed. Females were offered 3 mL (0.1 fl oz) of sterile defibrinated sheep blood (HemoStat Laboratories, Dixon, CA) for two consecutive days for egg development. Individual oviposition chambers were prepared by adding 50 mL (1.7 fl oz) dechlorinated tap water to 148 mL small plastic vials (5 oz/40 dram) lined with coffee filter material trimmed to 10.5 cm (4.1 in) diameter circles. Females were transferred to individual chambers and held there for six days to allow oviposition. After removing the mosquitoes, filter papers were dried out for six days in the insectary under the same conditions. To quantify larval hatch rates, dried filter papers were examined

for egg counts and deformities and then submerged in dechlorinated tap water. Larvae were fed ground fish food pellets (Hikari Cichlid Gold mini pellets, Hikari Sales U.S.A. Inc, Hayward, CA) and counted four days after water was added.

Pre-Release Planning

Based on results from the longevity and fecundity studies, NWMVCD decided to move forward with field releases of sterile male mosquitoes. The release site, a neighborhood in the McVicker Canyon area of Lake Elsinore, CA, was selected because of historical surveillance data and mosquito service requests indicating the presence of *Ae. aegypti*. In addition, this area has a natural mountainous border to the west, the Northern Cleveland National Forest, which would presumably prevent immigration of wild *Ae. aegypti* into the release area from that direction (Gato et al. 2021). Three 5-acre treatment plots (Plot A, Plot B, and Plot C) were designated as release areas (Figure 1). One 5-acre area approximately 1.6 km (1.0 mile) away from the treatment areas was designated as the control (Figure 1). BG-Sentinel 2 traps (n = 3 per plot) were deployed weekly for 4 weeks prior to the sterile male releases. In addition, prior to the first release, educational flyers were disseminated to the residents in person during door-to-door efforts and posted on NWMVCD social media accounts.

Sterile Male Releases

The irradiated sterile “Cali Strain” males purchased from Rad Source were shipped weekly for eight consecutive weeks under temperature-controlled conditions from Atlanta, Georgia. Upon arrival at the NWMVCD, mosquitoes were sorted into 15 rearing cages per release (n = 5 cages per plot). For releases 1–4, BugDorm cages (MegaView Science Co., Ltd., Taichung, Taiwan) were used, while releases 5–8 utilized mesh butterfly cages (RESTCLOUD Insect Habitat Cage Terrarium). Each cage contained approximately 2,000 mosquitoes and was provided with 10% sucrose solution. Cages were held overnight in the insectary under controlled conditions (25°C, 77% relative humidity) prior to release. The following morning, cages were transported to release sites in an enclosed, temperature-controlled vehicle. Releases were conducted by walking along predetermined transects within each plot while partially opening the cages to allow mosquitoes to disperse, thereby promoting an even distribution across the treatment areas. Mosquitoes that did not fly out of the cages on their own within ten minutes were retained in the cages, returned to the district facility, and frozen for subsequent counting to estimate release mortality. Population monitoring was conducted using BG-Sentinel 2 traps (n = 13), deployed five days after each weekly release. Following the eighth and final release, post-release surveillance continued at the same 13 trap locations on a weekly basis for an additional seven weeks.

Statistical Analysis

Data were analyzed with R (version 4.5.2) (R Core Team 2025). For the longevity study, R package *survival*

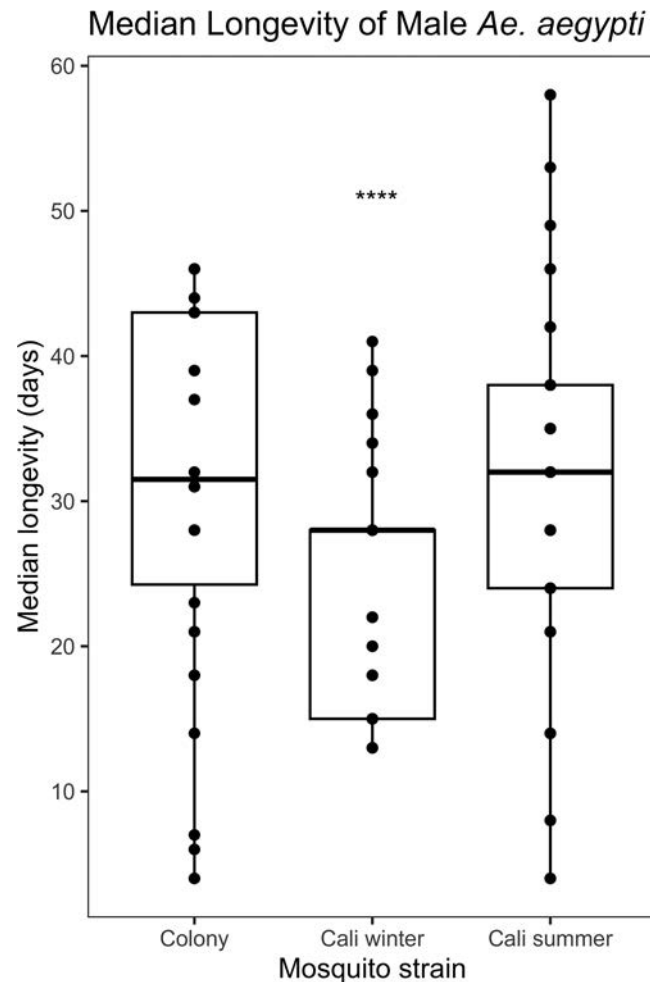


Figure 2.—Median longevity of three strains of male *Ae. aegypti* mosquitoes maintained under controlled laboratory conditions. “Colony” mosquitoes were not irradiated (n = 42), whereas both “Cali” strains were irradiated at 60 Gy by Rad Source prior to shipment to NWMVCD (n = 58 for Cali winter; n = 67 for Cali summer). Medians were compared by log-rank test.

(Therneau 2026) was used to calculate Kaplan-Meier survivorship curves and compare median longevity using a log-rank test between the three mosquito strains. Wilcoxon rank sum test was conducted to compare the hatch rates between colony and “Cali” strains. Generalized Linear Models (GLMs) were used to assess the adult abundance dynamics.

Results

Longevity

Median longevity for the three strains of male *Ae. aegypti* was: non-irradiated colony males, 31.5 days; “Cali strain” winter, 28.0 days; and “Cali strain” summer, 32.0 days. A log-rank test indicated a statistically significant difference in survivorship among the three groups ($\chi^2 = 32.9$, $d.f. = 2$, $n = 167$, $p < 0.001$) (Figure 2). Despite this statistical significance, the maximum difference in median longevity

Table 1.—Egg and larval counts from females mated to either “Cali strain” irradiated males or “Colony” non-irradiated males (n = 10 females for each group).

Strain	Eggs					Larvae	
	Total	Min	Max	Mean ($\pm$ SE)	Deformed (%)	Total	Hatching Rate (%)
Cali	485	0	102	48.5 ($\pm$ 13.8)	100	0	0
Colony	800	58	95	80.0 ($\pm$ 3.7)	1.5	385	48.3

among groups was only four days, and all strains exhibited a minimum median lifespan of at least four weeks. Therefore, these differences were considered biologically negligible.

Fecundity

Females from both mating chambers laid eggs (Table 1 and Figure 3), except for four females from the “Cali” chamber. All eggs from “Cali” chamber females were visibly deformed, whereas those from the “Colony” chamber were mostly normal (only 1.5% deformed). A nonparametric Wilcoxon rank sum test on the median egg counts of each group of females did not show any significant differences between fecundity of the females from the two chambers ($p = 0.15$). Larval counts were significantly different, with zero larvae hatching from the “Cali” chamber females and 48.3% of the eggs from the “Colony” chamber hatching (Table 1, Wilcoxon rank sum test, $p = 0.000064$). These results confirmed that the “Cali strain” males shipped from Rad Source were sterile and therefore could be released in the field without concern.

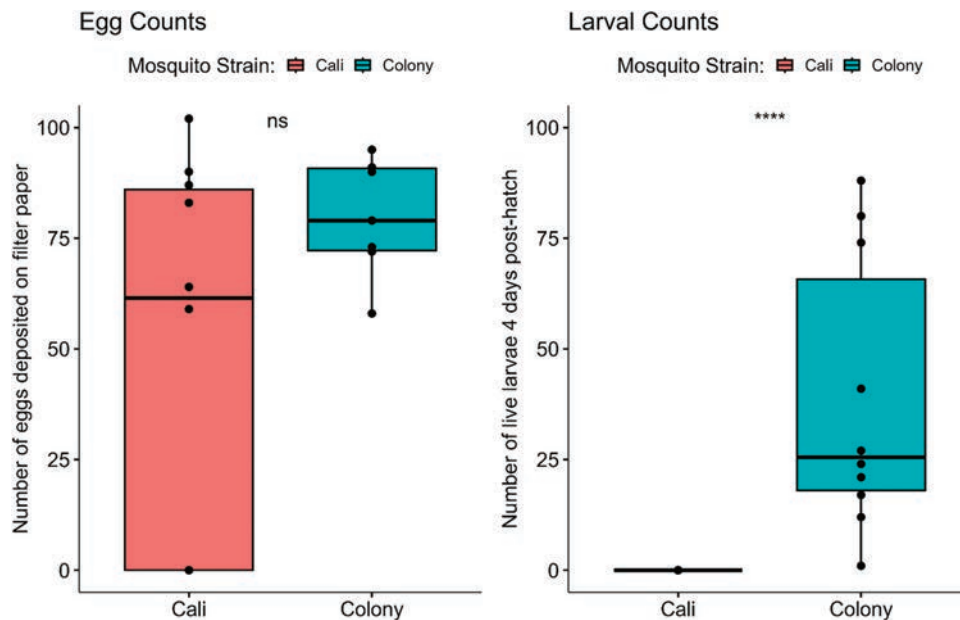
Field Releases

An average of 24,011 sterile male *Ae. aegypti* mosquitoes were released per week, with a mean mortality rate of 14.1%

(range: 4.9–26.8%). In total, 192,090 mosquitoes were released out of 224,000 received from Rad Source. A total of three dusted sterile males were recaptured in same BG-Sentinel 2 trap at Plot C.

Surveillance results did not indicate a clear difference in abundance between control and treatment plots (Figure 4). A total of 161 trap-nights yielded an overall mean of 3.1 female *Ae. aegypti* per trap-night over 19 weeks, across treatment plots A, B, and C. In the control plot, 63 trap-nights resulted in a higher overall mean of 4.6 females per trap-night. As an additional comparison, district-wide BG-Sentinel 2 trap data (excluding SIT study traps) showed an average of 3.7 females per trap-night ($n = 715$).

A generalized linear model (GLM) indicated a statistically significant difference between the combined treatment plots (A–C) and the control plot ($p = 0.0007$), with lower female abundance observed in treatment areas (intercept = -0.58). However, when accounting for temporal phases (pre-release, release, and post-release), the difference between treatment and control plots was no longer statistically significant ($p = 0.15$). Additionally, no significant differences were detected among the three experimental periods ($p = 0.83$).

**Figure 3.**—Egg and larval counts resulting from females mated to either non-irradiated Colony *Ae. aegypti* males (1:1 ratio, 10 adults of each sex in one chamber) or irradiated “Cali” *Ae. aegypti* males (1:1 ratio, 10 adults of each sex in one chamber). Egg counts were compared using Wilcoxon rank sum test between groups.

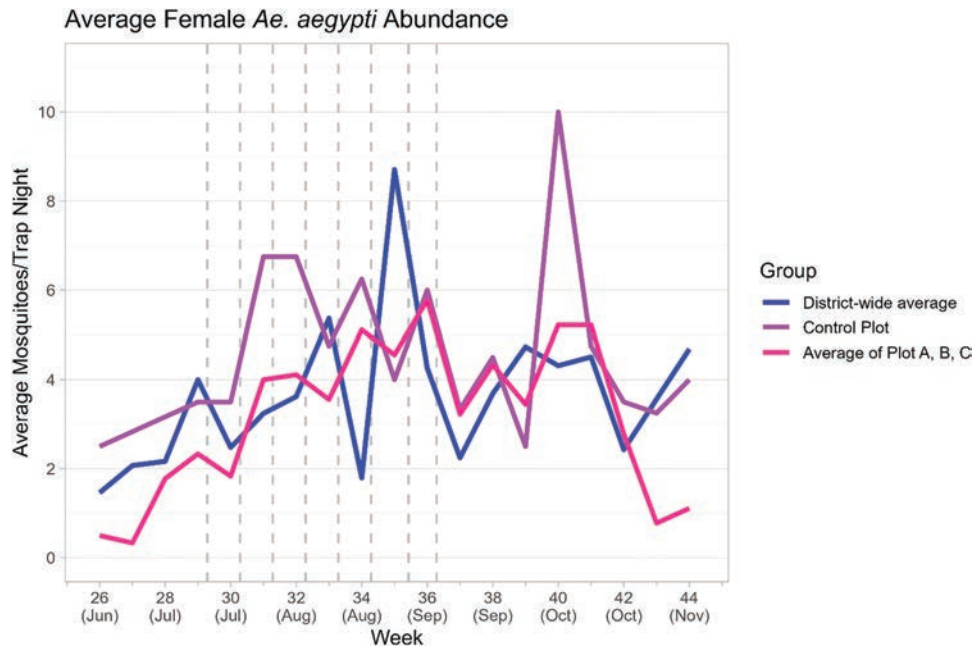


Figure 4.—Average female *Aedes aegypti* caught in BG-Sentinel 2 traps during the study period. Vertical dashed lines indicate sterile male releases. The district-wide average (blue line) does not include any traps in the SIT release area or Control area.

Discussion and Conclusion

Although the results did not demonstrate a clear effect of sterile male releases on the abundance of the target mosquito population, the study provided important operational insights for the NWMVCD. Repeated field releases highlighted several opportunities to improve release cage design and deployment efficiency.

During the initial release period (weeks 1–4), BugDorm cages were used due to their availability within the insectary. However, these cages proved cumbersome in the field and were prone to damage, largely due to the need for repeated disassembly for cleaning and reassembly each week. To improve operational efficiency, lightweight mesh butterfly cages were subsequently adopted and modified for mosquito release. Modifications included hand-sewn fabric sleeves (using the same material employed in BugDorm maintenance within the insectary) attached to the smaller lower zipper opening used for transferring sugar-water vials and dispensing mosquitoes from shipping containers. In addition, internal fabric seams were sealed with hot glue to prevent mosquitoes from becoming trapped in fabric folds. Because the butterfly cages feature a fully unzipped top, releases were conducted by partially opening the upper flap, allowing for more controlled dispersal of mosquitoes. Overall, these modified cages were easier to transport and allowed more consistent release rates of sterile males, thereby streamlining field operations.

This project demonstrated that the Sterile Insect Technique (SIT) can be a practical, scalable, and accessible tool for mosquito control programs lacking dedicated sterile male production facilities. Although overall *Ae. aegypti* abundance was low (< 4 females per trap-night) and

surveillance data were variable, preliminary findings indicated the operational feasibility of using outsourced sterile males as a cost-effective alternative to in-house rearing and irradiation.

Acknowledgements

We would like to thank Northwest Mosquito and Vector Control District staff for their contributions to mosquito surveillance, field releases, outreach efforts, and data collection. We also acknowledge the residents of McVicker Canyon in Lake Elsinore, for their cooperation and granting access during field operations.

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A preliminary examination of Incompatible Insect Technique (IIT) for control of *Aedes aegypti* within the Turlock Mosquito Abatement District in 2025

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Abstract

The Turlock Mosquito Abatement District (District) was established in 1946 and is tasked with providing mosquito control to the 966 square mile area of Southern Stanislaus County in the Central Valley of California. In 2019, the District made its first detection of the invasive mosquito *Aedes aegypti* in Stanislaus County. Following this initial discovery, *Ae. aegypti* quickly became established throughout the county and can now be found in all suburban locales. This has resulted in a dramatic increase in service requests resulting in the District exploring novel methods to control *Ae. aegypti* within the District. The biology of this mosquito and its preference for small cryptic larval sources renders traditional mosquito control methods ineffective and inefficient. One novel approach that has shown some success in controlling these mosquitoes is the Sterile Insect Technique (SIT), or more specifically in this case, the Incompatible Insect Technique (IIT). Male mosquitoes infected with *Wolbachia* are released regularly in a treatment zone and when mating with non-infected females the resulting eggs are non-viable. The District tested a 25-acre plot in south Turlock where only IIT was utilized as a control method. Infected male mosquitoes were shipped twice per week from Mosquito Mate Inc. and released at a rate of 1,200 male mosquitoes per acre, resulting in a total of 480,000 mosquitoes released over the 16-week release period. Results indicate that IIT alone may be adequate to control a population of *Ae. aegypti* in a localized area. Although while not eliminating the mosquito, population levels were reduced to below District control thresholds. Further development of this control strategy will need to occur to evolve IIT into more than just a novel technique, most notably by significantly reducing application costs and increasing the efficiency of large-scale applications.

VectorSurv tools for stronger communication

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Introduction

As a part of our mission to provide mosquito and vector control agencies with the tools they need to manage and prevent the spread of vector-borne diseases, VectorSurv provides tools to facilitate communication of results to key decision-makers within each agency and to allow agencies to connect with the public. Recent updates to VectorSurv have improved how agencies can manage testing notifications and provided a public-facing tool for California residents to find their local vector control agency. Our presentation provided an overview of these new and related existing tools within VectorSurv.

Methods

The VectorSurv team identifies and defines development requirements based on input from the CalSurv Steering Committee, member agencies of the Mosquito and Vector Control Association of California, and other state and local vector control and public health agencies. During the past year, we have updated the notifications management interface to allow agency managers to control which users receive notifications of new test results, a California vector control agency finder, and enhancements to publicly available VectorSurv maps.

Results and Discussion

Automated email notifications are generated by VectorSurv in response to specific events, such as receipt of samples at the DART laboratory or entry of new test results. With the new notification management interface, agency managers can easily see and control which users are eligible to receive notifications to ensure that the emails get to those

who need to see them. Users to whom permission is granted by the agency may then opt to receive notifications for each type of event. VectorSurv's user management tools allow agencies to assign a role such as View Only, User, or Agency Manager to each user. Each role allows a different level of access to the agency's data within VectorSurv. Assigning and regularly reviewing user roles is important to ensure that users have an appropriate level of access. VectorSurv recommends reviewing the users and assigned roles at least once per year to ensure that everyone has the correct level of access.

The Agency Finder is a public-facing map that allows visitors to identify and contact an appropriate vector control agency based on their geographic location or an address search. It displays agency boundary and contact information stored within the VectorSurv system, providing the public with a way to contact the relevant service provider. VectorSurv also provides other public-facing maps that display aggregated surveillance data and model-based risk estimates. These maps can be shared in public communications or embedded on a website, providing agencies with a way to communicate their work with the public in a manner consistent with their other messaging.

Acknowledgements

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Incorporating VectorSurv's new service visits and integrated data view modules into your response toolkit

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Introduction

VectorSurv (VectorSurv Development Team 2026) provides a range of data management and analysis tools used by vector control agencies for the surveillance and control of vectors and vector-borne pathogens. To expand the tools available to match agencies' workflows, VectorSurv has developed new features that allow agencies to track and manage their field work, such as responding to service requests about mosquitoes or other arthropods from the community. VectorSurv also has released a new map-based data display that allows agencies to interact with and manage their arthropod collections, pools, test result records and their pesticide application records. This presentation provided an overview of these tools, including VectorSurv's new Service Visits module and a new universal data viewer known as Integrated Data View.

Methods

The VectorSurv team defines development priorities by working with the CalSurv Steering Committee, state and local public health agencies, and mosquito control districts in the Mosquito and Vector Control Association of California. Projects are prioritized based on the value they provide to the vector-control community, development effort required, and scheduling constraints.

For 2025, the ability to capture additional types of field work was identified as a high priority by our MVCAC partners. VectorSurv has offered the ability to manage data related to mosquito and other arthropod trap collections and pesticide applications for many years, but prior data modules had not included a dedicated interface for recording field inspections where collection traps or pesticide applications may or may not have occurred, such as those in response to community mosquito complaints or as part of a human disease case investigation. In these situations, an agency may need to record other interventions beyond pesticide application, such as community education or source reduction.

Priorities during 2025 also included development of a tool that allows agencies to easily view and manage multiple data types at once. Because of their spatio-temporal

relationships, agencies need the ability to view and filter mosquito and other arthropod collections and pools, pathogen test results, pesticide applications, and service visits in a unified tool with paired spatial and tabular views of the data. This provides a more complete picture of the services that they have provided for the community and help to identify gaps in their coverage.

Results and Discussion

The Service Visit module allows agencies to collect and manage their data related to the work they have performed in the field. This module allows users to provide a name for the service performed, record the staff members who performed the service, and store the date and time that the field work was completed. The agency can record the location of the service visit by entering the coordinates of the location, searching for an address, or dropping a pin on a map within the web form. The module also allows the agency to flag particular service visits within the system to indicate that the service visit has not yet been completed or that there is a particular concern related to that service visit, which can be explained in the comment section.

Once saved, the Service Visit module enables the agency to add new data related to the visit or link to preexisting data that should be associated with the service visit record. Existing arthropod collection records of any type can be linked to service visits, such as a trap collection that was initiated at the time of the visit. Some collections, such as larval dips or backyard inspections, can be added directly from the service visit form when creating the service visit to make it easy to record collections that are often associated with this type of field work. It is also possible to record or link any pesticide applications that were performed during the service visit.

Although the Service Visit module focuses on the entry and association of field data, the Integrated Data View serves as the comprehensive management hub for these various data streams. The Integrated Data View provides a centralized interface for users to visualize and manage multiple VectorSurv record types simultaneously. Currently, it integrates arthropod collections, arthropod pools, pesticide applications, and service visits into a unified display

consisting of an interactive map and a corresponding data table. VectorSurv plans to add additional record types in the near future, such as sites and tick collections. The interface features an adjustable divider, allowing users to drag and resize the map and table panels as needed to allow the user to optimize the view for their screen size and needs.

To streamline data discovery, the Integrated Data View includes a date filter and a data settings menu. Data settings allow users to toggle specific record types on or off to customize the scope of their analysis. The Table View organizes records into tabs by data type, and supports standard management features including column sorting, pagination, and a dynamic search field for filtering large datasets. Specialized filters are available for some data types to provide more granular analysis; for example, a user could filter the arthropod pool table to show only pools with definitive test results or pools that are positive for a particular pathogen. Integrated action buttons allow users to delete or edit records.

The map component of the Integrated Data View provides a spatial representation of the filtered records. Interactive map controls include a recenter tool to frame all data points, a geocoder for address-based navigation, and a view toggle to switch between street and satellite imagery. Users can interact with items on the map to view detailed information;

in instances where multiple records share a single location, a popup generates a tabular view of all data records at that point. To connect tabular and spatial data, clicking any row in the table automatically pans and zooms the map to the corresponding record, facilitating a seamless transition between data management and geographic visualization.

Acknowledgements

We thank our partners, especially the Mosquito and Vector Control Association of California and the California Department of Public Health, for their many essential and long-standing contributions to VectorSurv's mission to strengthen the evidence basis for vector-borne disease control. During 2025, VectorSurv's work was supported primarily by funding from the State of California and also by partial support from cooperative agreement 6NU50CK000632 to the American Mosquito Control Association from the Centers for Disease Control and Prevention.

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Early or Late? Evaluating Areawide Larvicide Application timing across mosquito growth stages

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Introduction

Delta Mosquito and Vector Control District (DMVCD) has traditionally relied on property inspections to control *Aedes aegypti* populations through source reduction. Although effective, this method is time consuming, labor intensive, and dependent on resident cooperation.

To address this problem, DMVCD implemented an areawide larviciding strategy using Altosid SR-20 (Wellmark International 2017) to compare the efficacy of May vs. June interventions. The goal was to determine if shifting the treatment earlier or later in the season could provide extended suppression of *Ae. aegypti* populations throughout the summer.

Methods

Field calibration trials were conducted in partnership with Central Life Sciences to determine sprayer swath width and pesticide deposition. Water-sensitive cards and DropVision analysis established an effective 150 foot treatment swath. Larval assay cups confirmed 80% adult emergence inhibition at that distance,

validating application efficacy under standard nighttime environmental conditions in the southern San Joaquin Valley of California.

For the primary study, six sites with historically high *Ae. aegypti* abundance were selected in Visalia, CA. Two sites received the May intervention (weeks 19–22), two received the June intervention (weeks 23–26), and two served as untreated controls. Altosid SR-20 was applied weekly for four weeks at a rate of 0.75 oz/acre using a truck mounted A1 Super Duty Sprayer for each intervention site (Fig. 1). Adult populations were monitored for 23 weeks (weeks 17–39) using five BG-Sentinel traps at each site, all baited with dry ice. Additionally, four larval assay cups were placed in residential backyards during each treatment to verify product dispersal and deposition.

Results and Discussion

The results from the larval assay cups showed a greater average percent of adult emergence inhibition in the treatment groups (May: 42.0%; June: 33.5%) compared to the control (10.7%). Adult mosquito trap



Figure 1.—Areawide larvicide application using Altosid SR-20 via a truck mounted A1 Super Duty Sprayer in a residential neighborhood located in Visalia, CA.

counts were analyzed in JASP (JASP Team 2025) using a generalized linear model (GLM) with a negative binomial distribution. Adult mosquito surveillance data revealed that the May intervention (28% reduction, $p < 0.001$) and the June intervention (21% reduction, $p = 0.001$) significantly reduced adult *Ae. aegypti* females relative to the control for the remainder of the surveillance period after the final treatment.

Our study confirmed that early areawide larvicide treatments were effective at reducing *Ae. aegypti* populations compared to untreated sites. Although early intervention successfully delayed and suppressed the seasonal peak (August) of *Ae. aegypti* populations, long term or more complete suppression will require supplemental vector management strategies or additional applications.

Acknowledgements

We would like to thank Samer Elkashef and Dan Killingsworth from Central Life Sciences for their assistance with calibration of water sensitive spray cards, larval assay cups, and equipment.

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Developing a stormwater BMP inspection and treatment program

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What Are Stormwater BMPs?

BMP (Best Management Practices) refers to anything from rumble strips at construction site entrances to massive infiltration and storage galleries under parks and commercial properties. The Stormwater BMPs inspected by the Greater Los Angeles County Vector Control District (District) are also called “manufactured treatment devices”.

These artificial structures manage stormwater and runoff by diverting, filtering, infiltrating, and/or detaining water. By changing water flow path, removing trash, hydrocarbons, metals, and organic material to improve water quality, gathering water and infiltrating it into soil, or holding it for re-use, the hundreds of designs share the same goal: managing water. However, the construction details differ.

Mosquito control professionals become involved because most of these devices are designed to hold standing water, often for extended periods. They also gather trash and pollutants, and many are in insulated underground vaults, providing a temperature-controlled environment regardless of season. That multiplies mosquito control problems.

Why Are They Needed?

The regulatory environment explains why so many of these devices are used and why their numbers are constantly growing.

A brief list of some of the major regulations include: the Federal Water Pollution Control Act, later called the Clean Water Act, which was the start of serious water quality control efforts. It led to the EPA's permitting system, the National Pollution Discharge Elimination System (NPDES), which determines the federal requirements for water quality standards.

California's initial focus was flood control, with getting this water to the ocean through rivers and flood control channels as quickly as possible the major goal. Drought years made water retention much more important, leading to more potential mosquito sources.

The Porter-Colone Water Quality Control Act is the major state California governing water quality. The State Water Resources Control Board then created the nine California Regional Water Quality Control Boards that locally certify BMP designs.

Another layer of regulation lies at the county and/or city level. Local requirements add enforcement penalties and provide incentives. For example, the 2018 Los Angeles County Safe Clean Water Program's creation spurred a massive increase in BMPs. It funds stormwater projects through a parcel tax based on square footage. Increasing a property's permeable area reduces the amount of tax and installing BMPs provides a credit that offsets some of the tax. Other jurisdictions have similar programs and rules affecting the type and number of devices.

Identifying, Finding and Classifying BMPs

A dedicated program for BMP detection, monitoring and treatment will benefit your District. Our District's program started in November 2018. The existing underground storm drain (USD) program, was inefficient for BMP treatment because most devices were located off the main storm drains in sidewalks, parking lots, planters, etc. A dedicated BMP programs' allowed the District to establish a route and a process to monitor all BMPs within a reasonable time.

New programs priorities are based on available funds, staff, and equipment. After starting with two full time staff, the District now employs three two-person teams during the season. Focus is on larger devices such as hydrodynamic separators and infiltration/detention chamber systems. Small devices such as catch basin and drain filter inserts are also BMPs, but there can be 100+ of these devices at some larger sites, so they're handled by other programs. Concentrating on the larger, more productive sources is the best use of available resources.

When identifying which BMPs to monitor, check if local agencies have helpful listings. A spreadsheet listing probable BMPs for just Los Angeles City was found at the start of our program. The 27,000 initial entries were reduced to just over 2,000 likely sources. A single, unified source listing all new or planned BMPs most likely will not be present, so be aware of new construction and label it for follow-up checks during slower periods. Our District has been adding about 400 new units to our system each winter by surveying.

After determining possible BMP locations, start identifying the types to check and how they work. Manufacturer websites, especially for larger companies such as Contech and Oldcastle, are very helpful and a great

starting place. They often have diagrams showing unit components, water flow, and function. Knowing how the devices work and what the parts look like helps when examining an inspection port and figuring out where and how to apply treatments. At the beginning everything just looks like a hole with filthy water and trash, but with enough experience, recognizing the types, brands, and manufacturers becomes automatic.

The yearly CASQA Conference is also a useful resource for information on BMP issues and access to vendor representatives.

Essentials of a Treatment Program: Physical Tools

Several tools are useful for inspecting BMPs. Our most important tool is our magnetic lid puller. Many of our units don't have a pick hole, so a magnetic lid puller is a safe and efficient way to open them. A J-hook is needed for some covers. We also use custom lid pullers for manholes with pick holes, but there are other ways to get those units open. Basic hand tools include: a socket set, breaker bar, wrenches, a sledgehammer, etc. A really bright flashlight is essential.

Essentials of a Treatment Program: Digital Tools

Once the physical tools are in place, a system to collect and record data on unit locations, details, and history is crucial. Our District started with a shared Google Spread Sheet and pen and paper inspection sheets that were cross-referenced with the existing app our USD program uses. That wasn't scalable, so our in-house developer created an app for the program. We're on the second generation of the App now and make small tweaks and updates as necessary.

A detailed history identifies the most problematic units. Instead of wasting time on units that stay dry all year or never produce mosquitoes, efforts can be focused on the productive units. About half of our District's units only need to be checked once a year because they never produce. The program rotates the timing of the yearly check year-by-year so that units are observed during different seasonal conditions. With 4,500 units or so in our District, this is a huge time-saver. Field conditions change, so dormant units sometimes later hold water and produce mosquitoes. About 2-3% of our inactive units reactivate, so that yearly recheck is worthwhile.

Essentials of a Treatment Program: Inspecting and Treating

Remember, when inspecting a unit, only a portion of the unit is visible through the inspection port. Sampling the

water in the unit for larvae and/or pupae can be helpful. The biggest issue our District encounters when inspecting is lack of maintenance, mostly due to personnel turnover. This leads to forgotten units with no inspections. Maintenance personnel often see us inspecting units and tell us that they didn't even know the units were there. Because most BMPs are on private property, it can be challenging finding the responsible party. Larger manufacturers have an Operations and Maintenance (O&M) sheet on their sites that people responsible for maintenance can reference.

For treatments, Sumilarv is an extremely effective larvicide. The active ingredient, Pyriproxyfen, binds to the unit components and debris and gives a much longer residual effect than the other products that we've tried. We have documented 3 or more months of control in some units. This reduces turnaround time for an inspection cycle to 3-4 weeks. Because the label allows for 4 treatments per year, we use 3, typically at the beginning, middle, and end of the season. For interim treatments Methoprene-based larvicides (Altosid P35s and Metalarv) topped with a larvicidal oil gives good results.

Conclusion

The construction of BMPs is conducive to mosquito production. The number of BMPs will only increase over time. A program specifically addressing BMPs allows for the development of knowledgeable staff and provides efficient control. Program essentials include: what to check, where they are, and how to approach them.

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Mark Hall, Environmental Program Manager
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Susanne Kluh, General Manager

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Mosquito control at The Living Desert Zoo And Gardens

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Introduction

The Living Desert Zoo and Gardens is an institution that has existed in Coachella Valley for over 50 years. It covers 1,200 acres, with just over 80 acres dedicated to the zoo's current incarnation. They maintain over 600 animals and are involved in more than 80 conservation projects across the world. Some of the biggest attractions of the zoo are their giraffe, rhino, and Mexican Wolf enclosures.

Collaboration between the Coachella Valley Mosquito and Vector Control District (District) and The Living Desert Zoo and Gardens (Zoo) began at the end of 2013, when District staff met with the Zoo's staff regarding a particular species of *Culicoides*. These vectors were transmitting hemorrhagic fever to the impalas at the zoo. This disease and transmission cycle are now known to be endemic to the area. Additionally, future issues would arise at the zoo, and the district would respond to assist with these vector-related incidents. One example included the transmission of blue tongue virus transmitted by biting flies which was affecting the Gray Wolves at the zoo. Another project the district has worked on was testing the use of In-2-Care traps to control *Aedes aegypti* at The Living Desert.

Methods

In 2015, the operations department began assisting the zoo with mosquito control. Originally, the Operations department had assigned a technician to oversee the zoo because the location was in proximity to the technician's assigned zone. In the past technicians were assigned to a certain geographical area to review and provide surveillance. These domains are referred to as zones. Today, the district no longer operates with zones, but instead uses an approach where technicians are assigned, on average, 30 sites per day. With the removal of zones and a need for consistent review of the area, the leadership in our department decided to categorize The Living Desert as a special project in early 2025. The Living Desert, now deemed a special project, was assigned to be overseen by our Program Coordinator and Lead Vector Control Technicians. Our goals, when approaching the zoo, were to re-establish connection with a representative of the zoo, review the entire zoo area, investigate which types of sources were producing mosquitoes, provide control, and relay information to the zoo so they could take part in the process of protecting

public health. Based on our trapping data we knew that the primary mosquito of concern was the *Culex quinquefasciatus* because of their prevalence in the area and their ability to transmit viruses such as West Nile Virus (WNV) and St. Louis Encephalitis (SLE) to the animals and people at the zoo. Additionally, *Ae. aegypti* is another mosquito of concern because they are abundant in the area. As of today, we have not found *Ae. aegypti* responsible for virus transmission in the Coachella Valley.

In our approach to providing control at the zoo, we planned to visit the Living Desert every other week, at least twice a month, for a duration of 2 to 3 hours. Once we found more larval habitat at the zoo, we decided to dedicate a whole day to working at the zoo. Approximately 6 hours are now dedicated to field work after breaks and drive time is eliminated from an 8-hour workday. There is a total of 33 sites, 15 of which were added in 2025 and 1 added in March 2026. The sites vary from catch basins, dry-wells, fountains, various types of ponds, and water features in animal enclosures. These locations become favorable larval habitat because the water sources stagnate. Stagnation occurs for many reasons, for instance in catch basins water gathers in the sump areas restricting flow, in fountains and ponds there is a lack of circulation, or there is a need for maintenance freeing the passage for water flow when sources become clogged. Due to the size of the zoo an electric golf cart is used to travel throughout the zoo to reach every site. An electric golf cart is provided by the zoo as they prefer to use electric vehicles so not to disturb the animals. Gas-powered equipment also is not used to make applications. Traveling on the golf cart is also an efficient way to cover all the sites in the zoo within a working day.

Communication is an important component in our approach when collaborating with the Living Desert. We constantly communicate the estimated time of arrival and departure with our contact, so the zoo is aware of our presence. This is important in case there is something going on at the zoo that may require special attention such as, high attendance of guests, multiple school field trips, ongoing maintenance practices, off-limit areas, and construction. Additionally, our contact is informed of sources created by rain events, so they may drain them and eliminate potential mosquito sources. Communication is key for safety; besides being careful with guests and staff, it is important to be careful with wildlife, the animals, and their space.

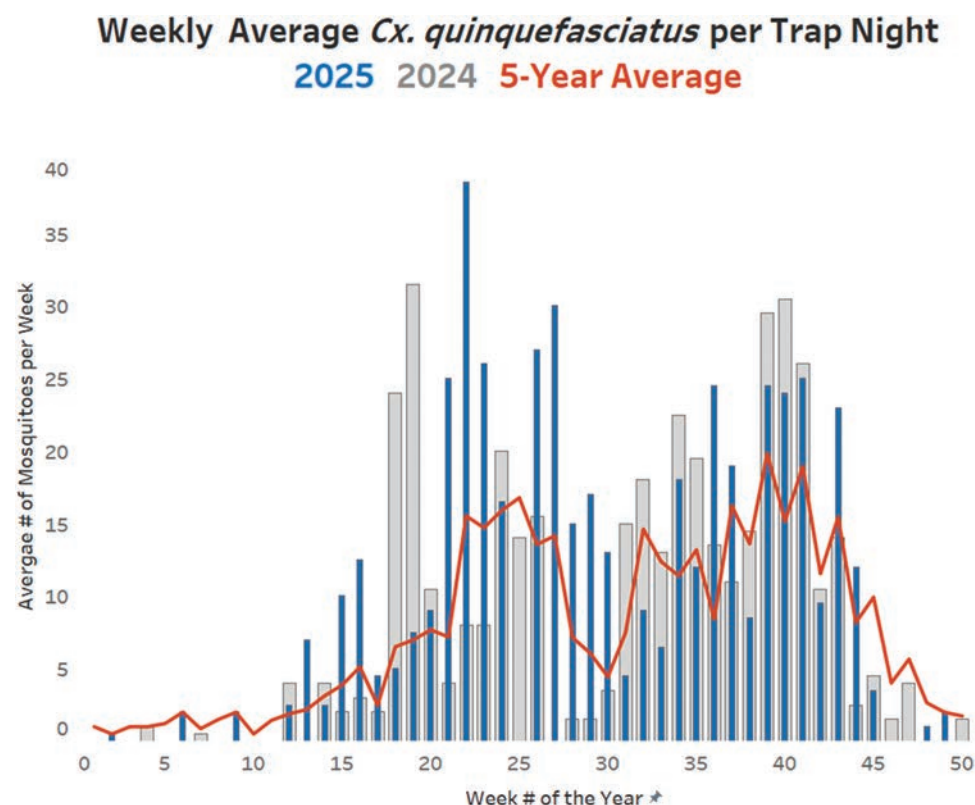


Figure 1.—Average number of *Cx. quinquefasciatus* adult females and males collected per trap night at the Living Desert Zoo and Garden during 2025, 2024 and previous 5 years.

Respecting the boundaries of these spaces is critical for all who are involved.

When performing inspections and treatments at The Living Desert, several items are carried in the golfcart to perform the work. Sampling devices such as a dipper, concentrator, drain pullers with “J” and “L” shaped hooks are taken. The different shaped hooks allow technicians to access different drains and grates with small or large openings at various weights. Measuring devices such as a scale, graduated cylinder, pitcher, and a measuring cup are used to accurately mix in the correct amount of chemical into my final solution. The B&G hand can is the only device that is used to apply larvicides. The B&G hand can is an ideal piece of equipment because of its portable size and ease of use in this type of environment and mode of travel.

Personal protective equipment and pesticides are the final materials taken into the field. In this collaboration we exclusively use Valent BioSciences’ Vectobac 12AS and VectoMax FG. Vectobac 12AS, *Bacillus thuringiensis israelensis* (BTI), at a rate range between 16 fluid ounces to 32 fluid ounces and VectoMax FG, *Bacillus sphaericus* (BS) and *Bacillus thuringiensis israelensis* (BTI), is used at a rate range of 10 pounds to 20 pounds per acre. The selection of higher rates is based on the conditions and parameters at the sites during sampling. Product labels are referenced when used. The reason for Vectobac products are being exclusively used is because The Living Desert’s Veterinary staff requested that we only use these products because of their special properties.

Results

The laboratory uses a combination of BG Sentinel traps and gravid traps to collect adult mosquitoes at the zoo. The laboratory’s surveillance efforts support our control efforts in operations by identifying the species of mosquitoes that are present. Another benefit from trapping is estimating the population abundance. Collecting data allows district staff to compare results before and after applications. Trap data is entered into VectorSurv and then extracted into the software application Tableau. The application, Tableau, allows our staff to view trap collection data. Data from Tableau can be referenced to determine if our control efforts are effective against mosquito populations at the zoo. When reading the charts for Figures 1 and 2, the x-axis indicates the number of trap weeks and the y-axis shows the average number of mosquitoes caught per night during that week. The grey bars represent the year 2024, while the blue bars compare data from 2025. The red line displays the five year average. When analyzing data in Figures 1 and 2, weekly average *Cx. quinquefasciatus* and *Ae. aegypti* per trap night show that control efforts were keeping the population below our emergency response thresholds of 80 *Culex* and 10 *Aedes* per trap night. Counts above these thresholds trigger attention for control efforts. In 2025 the highest counts for *Culex* were about 40 mosquitoes per night which is 50% below the maximum. For *Aedes* the highest counts were at 11 mosquitos per trap per night exceeding our threshold of

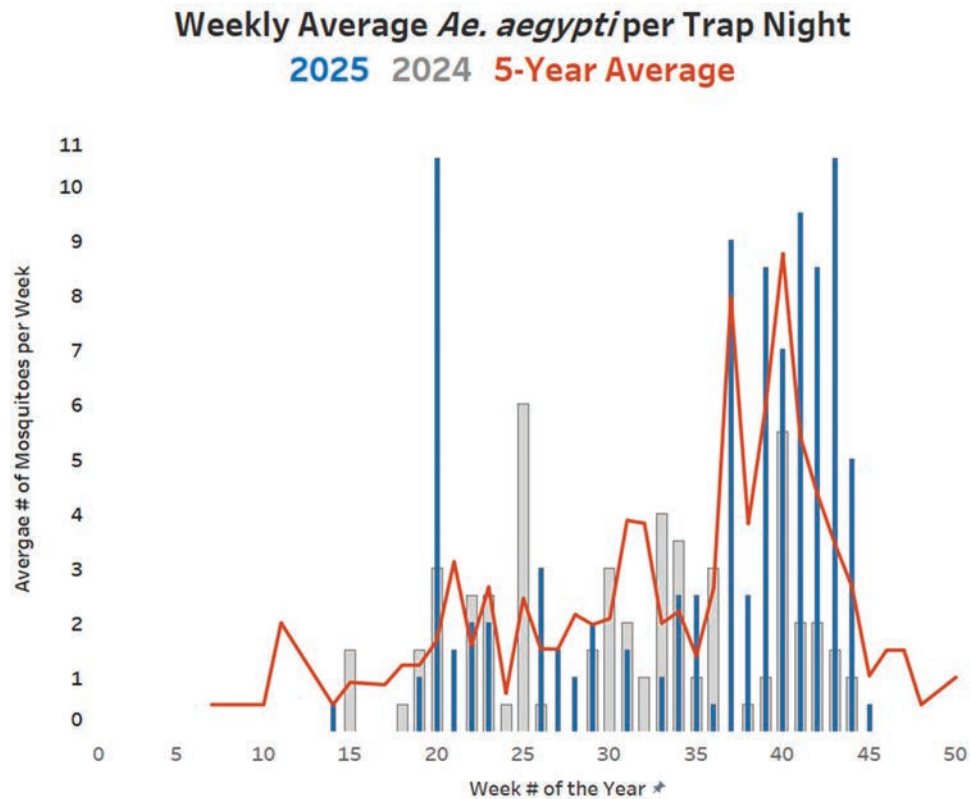


Figure 2.—Average number of *Ae. aegypti* adult females and males collected per trap night at the Living Desert Zoo and Garden during 2025, 2024 and previous 5 years.

10 twice during the year of 2025. Trap counts are a combination of male and female mosquitoes. Both charts show an increase in counts during fall. The theory is that during the late summer and early fall we had a lot of rainfall in the Coachella Valley.

Discussion

Our work with The Living Desert has continued and we are keeping good control over the area as trap counts remain low and under our thresholds. A couple of new sites have been added to our database system, while others have been taken care of by zoo staff. My goal has been to provide an operational breakdown of how we handle vector control in this type of environment. With support from the zoo, we will continue to be successful with our control. I have come to

learn that our strategy of one visit every other week is enough to provide quality service in the area. My hopes are to keep the mosquito counts low and reduce the number of treatments necessary at the zoo through alternate actions and methods of control.

Aknowledgements

I would also like to acknowledge our contact for the Living Desert, Heather Down, animal curator. Ms. Down was very helpful with this collaboration as she supported our mission. District staff, Alex Echenberg, Melissa Snelling, and Jennifer Henke were also contributors to this project. Their contributions helped me understand the history and relationship with the Living Desert. They supplied data from our trap and shared other helpful information.

Sending mosquitoes to their graves: Efficacy of Pyriproxyfen and Zinc-based interventions for mosquito control in cemetery flower vases

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Introduction

Cemeteries are important settings for mosquito control because they are typically located in urban areas and contain hundreds of funerary flower vases that provide perfect reproductive habitats for mosquito vectors, thereby increasing the risk for the spread of arboviruses (Vezzani 2007). In a previous study with cemetery vases, the Sacramento-Yolo Mosquito and Vector Control District (District) found success managing mosquitoes with an application of 5.0 grams of SumiLarv™, a pyriproxyfen-based larvicide, but also observed larval mortality in untreated, zinc-coated vases. Expanding on these findings, the current study evaluated the efficacy of using a reduced dose of SumiLarv and zinc-based treatments in inhibiting adult mosquito emergence from cemetery vases.

Methods

We conducted a field trial and three complementary laboratory bioassays, which included a matched bioassay, a zinc concentration assay, and a zinc coating assay. For the field trial, 100 galvanized steel vases were evenly divided into four treatments: bare vases with zinc exposed and no SumiLarv (VNS), vases lined with plastic floral bags and no SumiLarv (BNS), bare vases with SumiLarv (VS), and bagged vases with SumiLarv (BS). Vases were randomly distributed across a 134 m × 78 m cemetery plot (Fig. 1) and monitored weekly for mosquito presence and life stages, with pupal samples collected to assess emergence.

Matched laboratory bioassays were performed in triplicate with 20 laboratory-reared, CQ1-strain *Culex quiquefasciatus* per treatment type, with the addition of

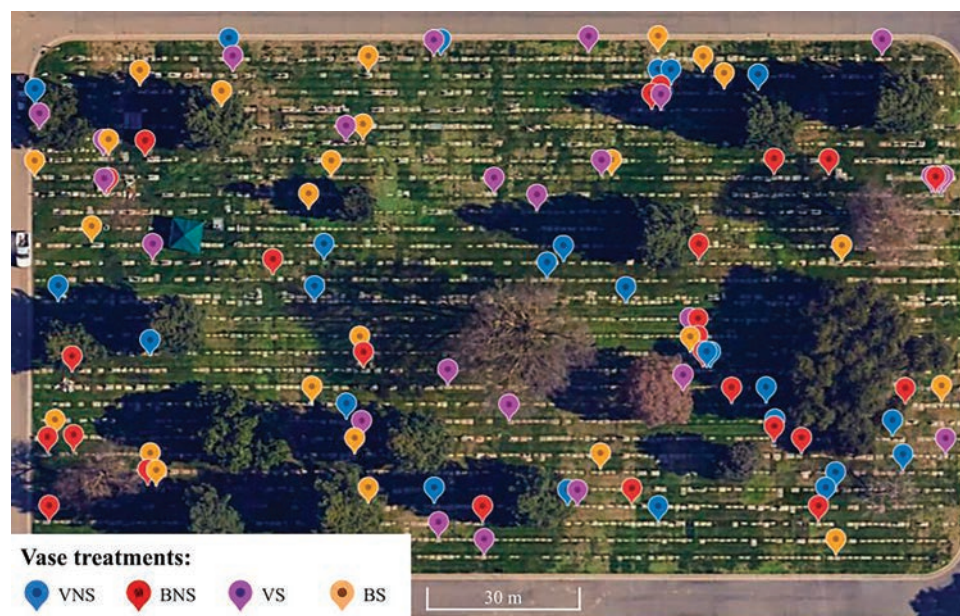


Figure 1.—Distribution map of vase treatments in an isolated cemetery plot at the East Lawn Elk Grove Memorial Park. Treatments were: bare vases with zinc exposed and no SumiLarv (VNS), vases lined with plastic floral bags and no SumiLarv (BNS), bare vases with SumiLarv (VS), and bagged vases with SumiLarv (BS).

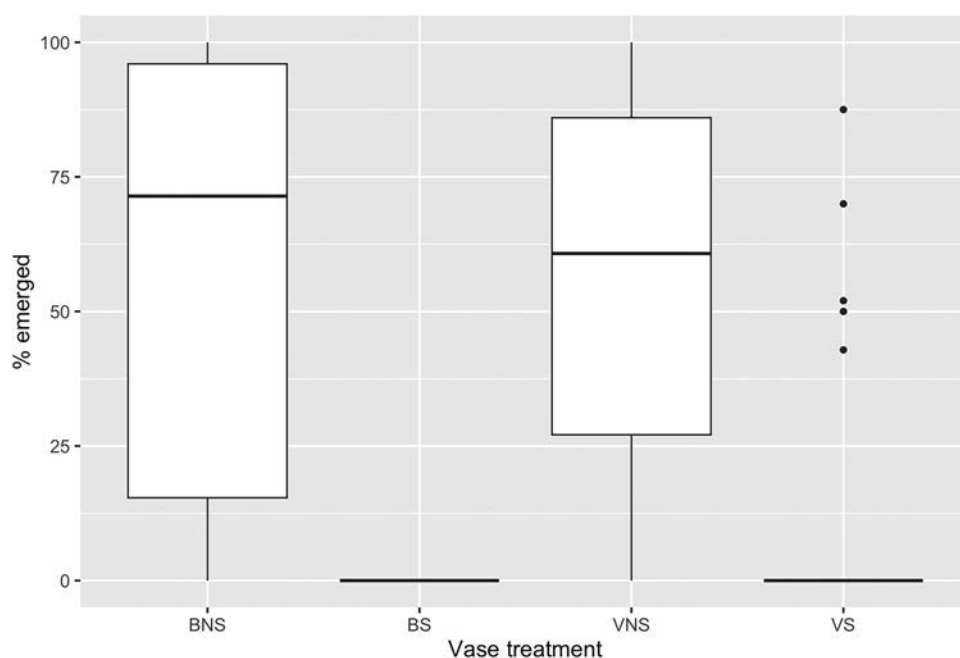


Figure 2.—Boxplots of percent adult emergence from collected pupae by vase treatment during the field trial. Treatments were: bare vases with zinc exposed and no SumiLarv (VNS), vases lined with plastic floral bags and no SumiLarv (BNS), bare vases with SumiLarv (VS), and bagged vases with SumiLarv (BS).

SumiLarv treatments with biweekly water replacement (VSW and BSW) to test for persistent effects. For the zinc concentration assay, CQ1 larvae were exposed to 0.0, 0.5, 1.0, 2.3, 2.5, and 5.0% Zn solutions in triplicate cups with 20 larvae per cup, and adult emergence was recorded

every 24 hours. For the zinc coating assay, egg rafts were taken from Sebring and CQ1 strains of *Cx. quinquefasciatus*. The number of eggs per raft were estimated under a microscope before hatching, and then one raft was placed in a cup that either contained no

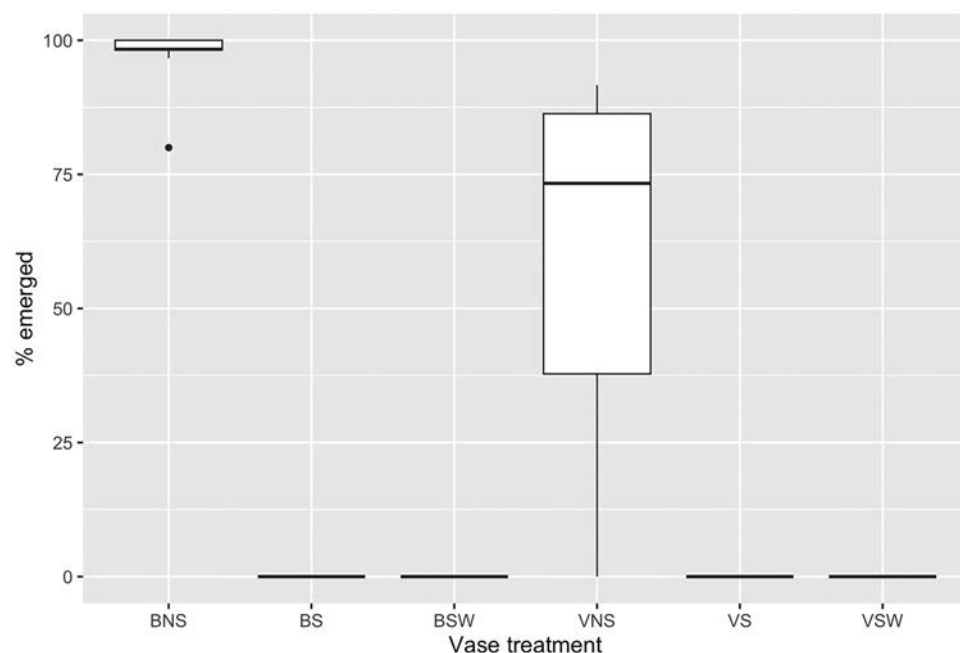


Figure 3.—Boxplots of percent adult emergence by vase treatment in the matched laboratory assay. Treatments were: bare vases with zinc exposed and no SumiLarv (VNS), vases lined with plastic floral bags and no SumiLarv (BNS), bare vases with SumiLarv (VS), bagged vases with SumiLarv (BS), bare vases with SumiLarv and biweekly water changes (VSW), and bagged vases with biweekly water changes (BSW).

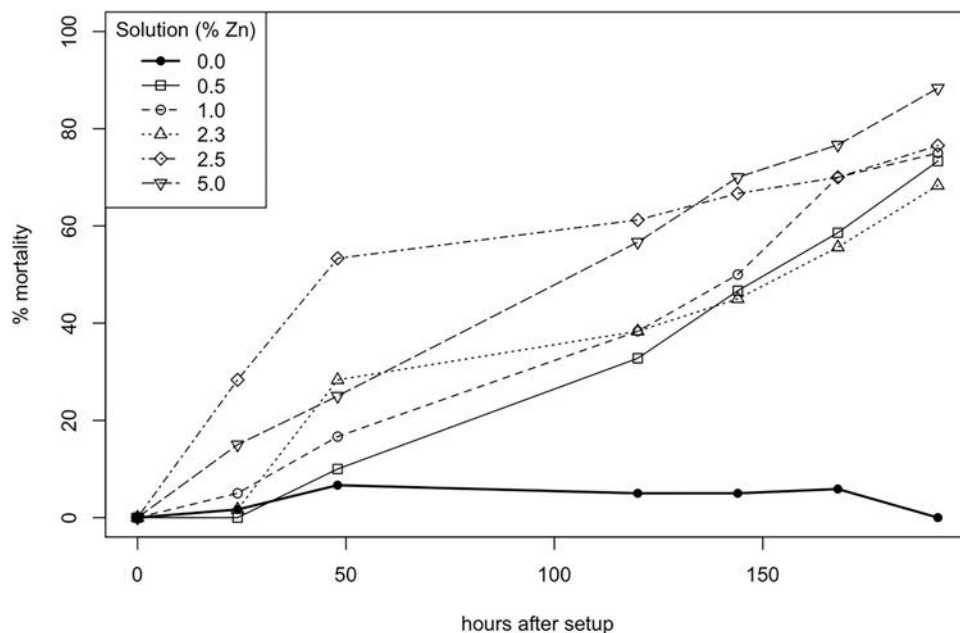


Figure 4.—Average percent larval mortality for different zinc concentrations plotted as a function of time of exposure.

hardware, or a washer or hexbolt made of stainless, hot-dip galvanized, or zinc-plated steel, for a total of 14 different treatments, done in triplicate. Emerging adults were counted every 24 hours to estimate the emergence rate.

Results and Discussion

Vases treated with SumiLarv persistently showed reduced rates of adult emergence compared to untreated vases in both the field trial and matched laboratory

bioassay (Fig. 2, Fig. 3). In non-SumiLarv treated vases, the bioassay showed significantly reduced emergence in bare vases compared to bagged vases. The field trial did not reflect this pattern, but results may have been impacted by other variables such as tree cover and public interference. There was a positive correlation between zinc concentration and larval mortality (Fig. 4), and all concentrations of zinc resulted in no adult emergence. Exposure to either galvanized or zinc-plated steel resulted in reduced emergence compared to the control treatments, with CQ1 appearing more susceptible than Sebring (Fig. 5).

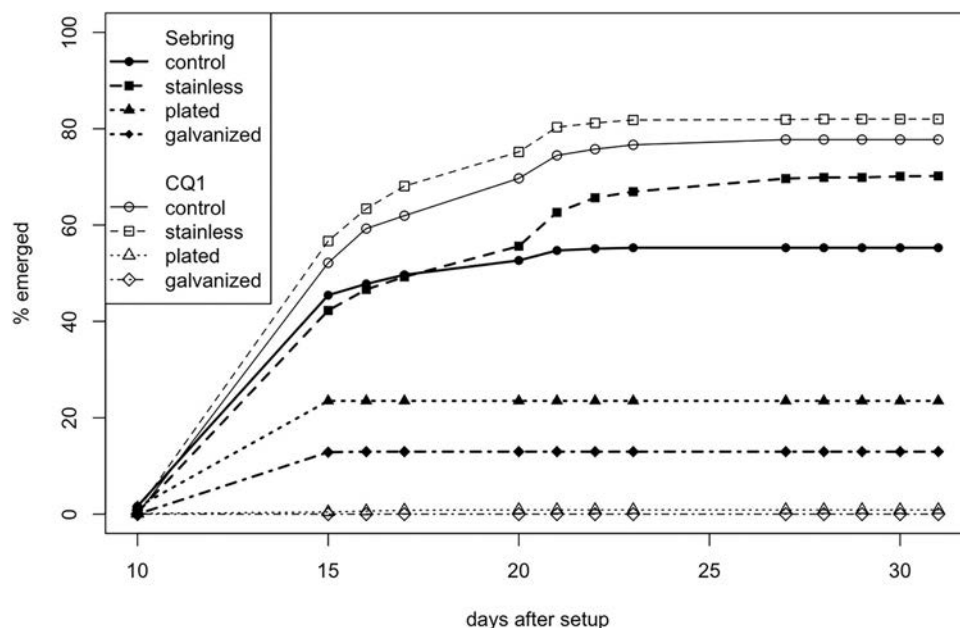


Figure 5.—Average percent adult emergence of 2 strains of *Cx. quiquefasciatus*. plotted as function of time of exposure to different zinc coatings.

Conclusions

We determined that 1.0 gram of SumiLarv per vase was effective in significantly reducing adult emergence, and that zinc-based intervention caused independent levels of mortality in mosquito larvae.

Acknowledgements

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A Dip in Time Saves Nine: A lean approach to mosquito control

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Abstract

Mosquito control programs aim to reduce mosquito populations, yet much of the work still centers on responding to production sources once they are found. Technicians identify and treat larval sources to prevent adult emergence, but the same sites often produce again because the conditions that created them remain unchanged.

This presentation frames mosquito production as a causal pathway that moves from upstream drivers and facilitators to sources, larval production, adult mosquitoes, and public health or nuisance signals. It describes how Delta Mosquito and Vector Control District has applied this approach operationally by treating service requests, surveillance data, and field observations as signals that generate leads for reconnaissance. Field staff then confirm or refute production, document sources and contributing conditions, and use both positive and negative findings as feedback to improve future targeting.

When productive sources are confirmed, interventions combine immediate control with source-focused prevention, including work with property owners or partners to change the conditions or behaviors that recreate mosquito production. The goal is fewer repeat sites, fewer repeat visits, and more durable population reduction. This presentation will outline the approach and describe how it has been incorporated into Delta MVCD operations.

From the laboratory bench to the sky: Improved ULV assays and drone ULV applications for better mosquito control

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Abstract

To control adult mosquitoes, selecting a product that is highly effective against the target species is a critical requirement. However, it can be hard to test products to see which are the most effective, because adulticide assays are difficult to perform and do not always provide the data that is needed. Field cage trials take a considerable amount of time and resources and can be greatly impacted by environmental conditions making it challenging to compare multiple products during a season. Other laboratory assays for adulticides, such as CDC bottle bioassays and topical applications, also can be hard to perform across a wide variety of active ingredients. These laboratory assays provide resistance data for active ingredients; however, they do not provide much information on which formulated products would be effective against target mosquitoes using a ULV application. To address these issues, the Salt Lake Mosquito Abatement District (SLCMAD) worked with University of Utah mechanical engineering students to create a spray tunnel to quickly test ULV applications in a laboratory setting with realistic spray parameters. After refining the prototype for a few seasons, staff at SLCMAD were able to use the spray tunnel to evaluate 10 products in 2025 and found which ones were most effective against the local *Culex tarsalis* population. This information was then used as SLCMAD evaluated off-the-shelf drones for ULV applications. The DJI T40 and T25 drones were both able to produce droplets of the correct size for aerial ULV applications. Spray trials with the two different drones were successfully conducted against caged mosquitoes, demonstrating another application method for adult mosquito control.

Vector control operations along an international border

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Abstract

San Diego County encompasses over 4,500 square miles and has more than 3.3M residents. Over 150 million people and vehicles cross from international locations into the county every year through a 60-mile-long land border with Mexico, two marine ports with both cruise and cargo ship traffic, and two international airports in San Diego and Tijuana MX. Through these ports, people, animals, and vectors cross daily and potentially introduce new diseases into San Diego County.

The County of San Diego Vector Control Program (VCP) monitors vectors in the border region including mosquitoes, ticks, and rodents. For example, in the border region, the VCP regularly checks at least 18 chronic mosquito sources and operates CO₂ mosquito surveillance traps at 8 locations every 1-2 weeks. This area is complex and under the jurisdiction of multiple agencies including the Border Patrol, California Fish and Wildlife Service, US Navy, CA State Parks, and County Department of Parks and Recreation. Regular communications are conducted with these agencies to form trusting relationships so that vector operations can proceed quickly and effectively within this region to control mosquitoes. These relationships are essential to tackle routine and complex problems in this area. For example, the exceptional winter storms of 2024 resulted in a berm break flooding of over 650 acres in the border region by sewage-contaminated waters crossing from Mexico. Enhanced monitoring of the affected area revealed elevated numbers of *Culex tarsalis* that exceeded normal levels by over 100X. Because the flood waters posed a potential health risk, both helicopter and truck-mounted equipment were used to apply larvicide to the flood water throughout the mosquito season to suppress mosquitoes and prevent potential amplification of WNV in the county.

Being an international gateway also presents challenges of frequent introductions of non-endemic diseases. For example, in 2024-2025 the VCP responded to 84 reports of dengue in travelers returning from abroad, over 50% of which had traveled to Mexico. The VCP responses included investigations and mosquito trapping at or near residences or other areas where the cases may have been exposed to mosquitoes, testing mosquitoes for viruses, and proactively treating neighborhoods if mosquito levels exceeded transmission risk thresholds.

Mosquito pesticide resistance levels in the border region are also assessed routinely so that the VCP can be prepared if it needed to respond to a disease outbreak. Fortunately, preliminary data in *Culex quinquefasciatus* indicated that the frequency of pesticide resistance alleles was lower in the border region than in more central parts of San Diego.

To enhance Outreach and community engagement in the border region, all vector educational materials and presentations are offered in English and Spanish. Geofencing is used in yearly educational media campaigns to reach people crossing the border and provide them with information for protection against vector-borne diseases. The VCP also partners with groups that serve border communities including community collaboratives and the Mexican consulate as well as with schools and libraries in the region.

Disease outbreaks across the border in Mexico inform VCP activities, such as tick surveillance in response to high levels of Rocky Mountain spotted fever (RMSF) in Mexico. Tick surveillance via flagging and collecting ticks from rodents captured for speciation and testing is performed year-round. Appropriate species are tested at the VCP in-house laboratory for tularemia, Lyme, and spotted fever group rickettsia, including RMSF. In addition, rodents are monitored at 6 international ports of entry twice a year to survey for invasive species as well as to detect plague and hantaviruses.

In conclusion, the VCP monitors the border region for invasive species and disease incursions and tailors outreach programs to reach international communities in the area. The VCP intervenes with control measures when necessary to prevent the potential spread of vector borne diseases. The region presents unique and complex challenges for vector control and surveillance and requires communication and collaboration with many agencies to conduct effective and efficient operations.

Droning on about the application and monitoring of large-source UAS treatments

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Abstract

Unmanned Aerial Systems (UAS) are increasingly used for pesticide applications due to their ability to precisely target treatment habitats, improve application efficiency, and access physically isolated larval habitats. However, mosquito habitats are rarely uniform—they vary in depth, temperature, vegetation, nutrient availability, light penetration, and surface matting. Despite advances in precision mapping, treatment plans often cover entire surface areas even though larval development is typically concentrated along pond margins or within shallow pockets. In addition, remote or unstable environments often limit pre- and post-treatment sampling to assess larval mortality.

The current study investigated whether UAS-based larvicide applications confined to pond margins could effectively reduce mosquito emergence compared to full-coverage treatments. A novel semi-field larval monitoring container was evaluated for its utility in assessing larvicidal activity under field conditions. Lessons learned from implementing and monitoring large-scale larvicide treatments were presented and discussed, providing insights for improving UAS application strategies and larval surveillance methods.

A real Buzzkill: When colonies refuse to cooperate

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Abstract

The growing need for mosquito colonies of several species has led to different housing, rearing, and feeding strategies over time. Despite mosquito ability to thrive in heat greater than 100 °F, with little humidity, combating Santa Ana winds, in some of the most polluted and/or nutrient rich waters around, indoor mosquito colonies seem to require very precise environmental conditions. Optimal rearing depends on careful regulation of temperature, humidity, photoperiod, water quality, and airflow to support larval growth and adult reproduction. Even under these standardized conditions, challenges with egg desiccation, larval food and rate of feeding, and contaminant exposure remain persistent threats to colony stability and scheduling for use, particularly when aligning with bioassay timelines or use in Sterilized Insect Technique programs. The current presentation highlights the key challenges encountered in establishing and maintaining mosquito colonies across different facility types and discusses the resource investments and logistical considerations required to scale up production.

How sampling for IGR field assays can easily ‘go off the deep end’

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Introduction

Greater Los Angeles County Vector Control District inspects and treats thousands of mosquito sources throughout its service area. Many sources cannot be tipped or tossed to drain, instead they must be treated with pesticides at regular intervals to prevent mosquito production. This type of preventive larval source management (LSM) strategy is a cornerstone of mosquito programs implemented by local government agencies, owing to the cost effectiveness of larval control and efficiencies offered by modern mapping and data-tracking systems that support inspection and treatment operations. However, the sheer abundance, size, and/or persistent nature of these recurring water bodies negatively impact District resources. Insect growth regulators (IGR) purport to have long residual efficacy which would reduce the number of treatments. Yet assays to test the residual efficacy of IGRs have been problematic. The aim herein is to describe sampling procedures, and report on issues with methodology and future goals for continued IGR efficacy assessments.

Methods

Trials with the IGRs Sumilarv 0.5G, MetaLarv S-PT, and MetaLarv XRP were conducted as applications to un-serviced swimming pools, underground utility vaults, and stormwater capture devices from 2020 to 2025. Sources with mosquitoes were treated and pupae were sampled weekly. Samples in 2020 were placed in glass jars with source water, whereas later trials used a Petri dish with a layer of filter paper at the bottom and

just enough source water to keep pupae moist until eclosion. One trial conducted with MetaLarv in stormwater capture devices sampled the water instead of pupae. Here, various susceptible strains of colony *Culex quinquefasciatus* larvae were added to the water samples and allowed to pupate. Pupae were given 5 days to eclose in all trials. Only adults that had completely emerged from the pupal exuviae were counted. Inhibition of emergence (%IE) was calculated using the formula $\%IE = 100 (C-T/C)$, where C represents the number emerged in the controls and T represents the number emerged in the treated samples. Sampling was conducted until the %IE fell below 80%.

Results and Discussion

Treated sources had a limited number of pupae present and in many cases, none were seen when sampling. Many sites without pupae remained that way for much of the trials. Where pupae were not collected, rates of inhibition couldn't be calculated using the aforementioned formula, because species absence is not equivalent to the inhibition of emergence. Because of the risk of WNV and the nuisance caused by leaving untreated sources in urban environments, field controls were not used, instead the trials relied on laboratory controls. Many samples had grown mold and bacteria before they could be read. Additional sampling issues included: sources too difficult to access for sampling, sources lost to follow up for various reasons, and contamination from other treated sources. Despite these difficulties, trials will be conducted again in the future with more robust controls and sampling methods.

