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Monday Plenary Session

Monday Reeves Award competition

Monday Walton Poster Session

Monday Afternoon Session A. Engaging a Diverse Popualtion

Monday Afternoon Session B. Natural Lands Symposia

Monday Afternoon Session B. 3D Printing

Tuesday Morning Session A. Vector control in an Urban Landscape

Tuesday Morning Session B. District Preparedness and Planning

Tuesday Morning Session A. Cyber Security

Tuesday Afternoon Session A. Managing mosquitoes in agricultural systems and impoundment waters

Tuesday Afternoon Session B. Unmanned Aircraft Systems

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 *Aedes melanimon* in the Sacramento Valley of California
1990	Gary N. Fritz	Polytenes, isozymes and hybrids: deciphering genetic variability in *Anopheles freeborni*
1991	David R. Mercer	Tannic acid concentration mediates *Aedes sierrensis* development and parasitism by *Lambornella clarki*
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 *Culex* mosquitoes
1994	Merry-Holliday-Hanson	Size-related cost of swarming in *Anopheles freeborni*
1995	Margaret C. Wirth	Multiple mechanisms cause organophosphate resistance in *Culex pipiens* from Cyprus
1996	No award	
1997	John Gimnig	Genetic and morphological characterization of the *Aedes* (*Ochlerotatus*) *dorsalis* group
1998	Yvonne Ann Offill	A Comparison of mosquito control by two larvivorous fishes, the stickleback (*Gasterosteus aculeatus*) and the mosquitofish (*Gambusia affinis*)
1999	Parker D. Workman	Adult spatial emergence patterns and larval behavior of the “Tule Mosquito,” *Culex erythrothorax*
2000	Jason L. Rasgon	Geographic distribution of *Wolbachia* in California *Culex pipiens* 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 *Culex* (Diptera: Culicidae) species
2004	No award	
2005	Troy Waite	Improved methods for identifying elevated enzyme activities in pyrethroid-resistanct mosquitoes
2006	Lisa J. Reimer	Distribution of resistance genes in mosquitoes: a case study of *Anopheles gambiae* 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 *Culex* mosquitoes in the Sacramento Valley, California
2013	Kimberly Nelson	The effect of red imported fire ant (*Solenopsis invicta* Buren) control on neighborhoods in Orange County, California
2014	Thomas M. Gilbreath, III	Land Use Change and the Microbial Ecology of *Anopheles gambiae*
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 (*Dirofilaria immitis*) Prevalence in Two Florida Communities
2018	Kim Y. Hung	House Fly (*Musca domestica L.*) Attraction to Insect Honeydew
2019	Matteo Marcantonia	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, *Gambusia affinis*, and their effect on the oviposition of *Culex tarsalis*
2021	Vanessa Hill	Evaluation of residential property types for *Ades aegypti* habitats in Placer County, California
2022	Phurchhoki Sherpa	Asian longhorned tick, *Haemaphysalis longicornis* (Ixodida: Ixodidae), and optimal collection methods for the tick in the Northeast United States
2023	Mark Dery	Effect of bed bugs (Hemiptera: Cimicidae) aldehydes efficacy of fungal biopesticides
2024	Mario Novelo	Dengue and chikungunya virus loads in the mosquito *Aedes aegypti* are determined by distinct genetic architectures

Presidential address: Challenges of Modern Mosquito Control

Conlin Reis*

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The 92nd Mosquito and Vector Control Association Conference took place from January 21 to January 24, 2024, at the Portola Hotel in Monterey, California. This year's theme, "Vector Control in the Modern World," highlighted the unique challenges faced by public health vector control in recent years and the industry's response to these changes. Four plenary speakers set the tone for the conference by addressing the history of mosquito control in California, contrasting it with divergent approaches in Africa, and highlighting real clinical cases along with effective communication techniques for modern audiences. Following the plenary session were the Reeves New Investigator Award competition and the William Walton poster competition.

The conference featured 86 talks, 1 panel discussion, and 17 poster presentations, including first-time symposia on 3D printing and cybersecurity. There was also a panel discussion on the first local transmission of dengue virus in California. Additional activities included a trustee training session, a 5k run, and an offsite awards ceremony and reception at the Monterey Bay Aquarium. Feedback for the conference was mostly positive, particularly regarding the new symposia and events.

The Challenges of Modern Mosquito Control

In recent years, the field of mosquito control has faced unprecedented challenges that test the adaptability and resilience of public health initiatives in California and beyond. Environmental changes and societal shifts have impacted both the scope of vector control operations and the complexity of the social and regulatory landscape in which they operate.

Environmental Changes

The reemergence of the historic Tulare Lake in the spring of 2023 exemplifies the extreme impacts that changing environmental conditions can have on public health vector control (CalOES, 2023). Excess precipitation and record snowpack resulted in thousands of acres of flooded land across the Central Valley. Inundated with mosquitoes, afflicted agencies relied on neighboring districts, state, and federal aid to prevent a potential public health crisis. Many vector control districts lack the resources to scale operations sufficiently to contend with extreme weather events and extensive flooding. Warming temperatures have further complicated control efforts by extending the distribution of invasive mosquito species, thereby increasing the risk of transmitting imported vector-borne pathogens (OEHHA, 2019).

Public Health Infrastructure and the Impact of the COVID-19 Pandemic

The global COVID-19 pandemic has brought unprecedented attention to public health infrastructure, emphasizing the importance of robust systems capable of addressing both pandemic and vector-borne threats. However, the pandemic also fractured public opinion concerning government interventions in personal liberties and health practices. This division poses a direct challenge to the implementation of vector control measures, which often require community cooperation and trust in public health directives. Vector control districts must dedicate considerable resources to gaining and maintaining public trust, especially when utilizing heightened interventions or new technologies. While the renewed focus on gaps in public health networks has created potential for increased funding and resources, districts must be prepared to utilize these resources transparently and responsibly.

Information Overload and Misinformation

The rise of independent media and social networks has led to an information overload, blurring the distinction between reliable and unreliable sources. Misinformation regarding vector control methods can hinder public health efforts, creating resistance to scientifically backed interventions and promoting ineffective or harmful alternatives. This perception problem is magnified by the introduction of new technologies, including genetic techniques that are prone to popular controversy (Mitchell, 2019). Districts have increasingly relied on communication specialists, recognizing that as important as the accuracy of public health messaging is, the ability to effectively communicate is equally crucial.

Legislative and Regulatory Challenges

Public officials, influenced by popular movements, have enacted legislative and regulatory changes that often come with unintended consequences for vector control. The legislative landscape can influence the availability and use of pesticides, water management practices, and the deployment of new technologies like drone-based applications. Advocacy in this area is critical to ensure that the specific needs of vector control programs are considered in policymaking processes.

Historically, mosquito control has faced the loss of key control products due to environmental concerns and associated regulation. Restrictions on pesticides and related operations have only expanded since then. With dwindling tools available for public health vector control, districts can no longer afford to remain inactive in the legislative and regulatory process.

New Species Introductions and Local Transmission of Imported Pathogens

The introduction of new mosquito species into California, such as *Aedes aegypti, Aedes albopictus*, and *Aedes notoscriptus*, poses significant challenges for vector control. These species are known for their aggressive daytime biting behaviors and potential to spread arboviruses such as dengue, Zika, and chikungunya. The nuisance pressure alone has increased the burden on local vector control districts, with increased public demand for service. Resources can be further strained by the need for rapid and comprehensive response to incidents of local transmission by invasive *Aedes*.

Recent local transmissions of dengue in Southern California underscore the evolving nature of vector-borne disease threats. As vectors adapt to new environments and expand their geographical range, public health systems must adapt quickly to manage these emerging health threats. Depending on the extent of the transmission event, vector control districts could find themselves unable to scale up their operations to the level necessary for an effective response.

The Two Pillars of the MVCAC

The Mosquito and Vector Control Association (MVCAC) plays a key role in enhancing local vector control districts' ability to deal with modern challenges. At its core, the MVCAC has two primary pillars: advocacy and collaboration.

Advocacy

Strong advocacy from leading mosquito control academics and professionals enabled California to enact one of the most comprehensive and flexible statutes for public health vector control in the nation. The MVCAC continues to focus on advocacy through its Legislative, Public Relations, and Regulatory Affairs committees, along with a professional advocacy team. Proactive advocacy on key issues has allowed California vector control agencies to maintain a protected place in legislative and regulatory frameworks and to implement new technologies. Through these efforts, public health exemptions have been added to pesticide restrictions, funding has been established for the statewide surveillance database VectorSurv, and a pathway to drone applications of public health pesticides was created (MVCAC, Leg and Adv, 2024). The association continues to engage in vital issues, seeking to align legislation and regulations with the challenges facing vector control districts.

Collaboration

The second pillar of the MVCAC, collaboration, ensures that vector control districts share knowledge, resources, and best practices to address common challenges. Collaboration is crucial in responding to environmental changes, public health crises, and legislative hurdles. By fostering a cooperative environment, the MVCAC helps districts to optimize their operations, innovate solutions, and effectively advocate for necessary resources and policies.

California's vector control districts vary significantly in size, ranging from small districts with limited staff and resources to large, well-funded operations. Through the MVCAC, even the smallest districts gain access to the collective resources, expertise, and innovations of larger districts. This collaborative network allows districts of all sizes to benefit from the latest technological advancements, regulatory insights, and best practices in vector control.

The MVCAC's numerous committees exemplify this collaborative spirit by focusing on specialized areas crucial to vector control. The Drone Committee works with both technology and regulations surrounding drone use, ensuring districts can safely and effectively deploy drones for surveillance and control operations. The Integrated Vector Management (IVM) Committee evaluates various techniques and prepares best management practices (BMPs) and standard operating procedures (SOPs), ensuring that districts implement the most effective and efficient control measures. The Laboratory Technology Committee assesses new technologies for vector identification and pathogen detection, facilitating the integration of cutting-edge tools into routine operations. The Vector Control Research Committee directs research into key areas, identifying gaps in current knowledge and spearheading initiatives to address emerging vector-borne threats. These are just a few of the many committees within the MVCAC that enable comprehensive collaboration and resource-sharing across all vector control districts in California. Through these efforts, the MVCAC strengthens the overall public health infrastructure, ensuring a robust and unified response to vector-borne disease threats.

Facing the Future

The body of work presented at this conference and throughout the year by the MVCAC's talented membership and the broader vector control community strengthens the industry as a whole against these modern challenges. With contributions not only to the science of vector control, but also to new technologies, public outreach, and operational preparedness, districts have a myriad of new resources to help them face our ever-changing world. I am grateful to every individual who dedicated their time and energy to making this conference a success.

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"By Their Deeds You Will Know Them." Matthew 7:16

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Introduction

It is an honor and a pleasure to address the 92nd Annual Meeting of the Mosquito and Vector Control Association of California. This organization has a rich and storied history. Ninety-four years ago, on December 16, 1930, thirty individuals gathered in Room 204 of Agricultural Hall on the University of California Berkeley campus. They described the meeting as a Conference of Superintendents and Trustees of Mosquito Abatement Districts in California. There had been, in fact, at least a half dozen earlier dinners and "occasional meetings" at the UC Faculty Club in the 1920s of individuals dedicated to the mission of establishing a state-wide anti-mosquito movement. In 1940, the Conference changed its name to the California Mosquito Control Association to reflect its mission and purpose. MVCAC's 92nd meeting is the latest representative of this tradition. I propose to spend a few minutes reflecting on what I will argue are the two most important of California's contributions to the American mosquito crusade: A commitment to science and a dedication to public service.

I would be remiss if I failed to express my gratitude to several individuals. I want to thank publicly Robert Washino and Bruce Eldridge for their kindness and sage counsel. Wherever I have gone in my research, the names Washino and Eldridge universally evoke respect and admiration: Respect for their commitment to the highest professional standards; and admiration for their collegiality. I am indebted to Dave Brown, Gary Goodman, Susanne Kluh, and Joel Buttner for their hospitality. Finally, I want to thank Conlin Reis for his kind invitation and Rachel Hickerson for her hard work in making this conference possible.

In a talk before a group of individuals who have built their careers on waging war against six-legged-blood-sucking pests, I should, in fairness, note that there is at least one instance of mosquitoes playing a vital role in state building. I am referring to the contribution of mosquitoes to David's rise to power as Israel's king. Some of you may recall the story of David and the slingshot. The episode I would like to call to your attention comes in the twenty-fourth chapter of First Samuel. Saul and his army are pursuing David through the wilderness of Enkedi. According to the Hebrew Scriptures, David seeks sanctuary in a cave. Later, Saul and Abner spend the night in the same cave. The next day, they depart, and David escapes.

Evelyn Mitchell, who was the first woman to write a book on mosquitoes and mosquito control, discovered a different account of David's encounter with Saul in the *Talmud*, the ancient collection of Jewish law and commentary on the Hebrew Scriptures. Mitchell, who earned both an MD and a Ph.D., recounts the *Talmud's* variation on First Samuel at the beginning of her 1906 *Mosquito Life: The Habits and Life Cycles of Mosquitoes of the United States, Methods for their Control*. The accounts are similar with one major difference. In the *Talmud*, David leaves the cave before Saul wakes. When David came to the cave's entrance, he found Abner asleep, sprawled across the opening as a sentinel against intruders. David gingerly steps over Abner, only to have Abner move one of his legs so that his ankle rests on David's foot. At that moment, Israel's fate was in jeopardy. The anonymous author of the *Talmud* reports that the "Lord sent a mosquito." The mosquito bit Abner who then moved his leg, allowing David to escape. David was saved and Israel's future was secured. Christians in this room will recall the opening verses of *Matthew*, Chapter 1, which declare, "From the house of David will come a Lord." Mitchell's story removes any doubt that you are biblically correct when you slap a mosquito and say "Jesus." (Mitchell 1906)

My objective is not to discuss the theology of mosquitoes. I propose to spend a few minutes looking backward at three episodes in the history of the American anti-mosquito movement. I hasten to add I am aware of what happened biblically to another person who glanced backward. My hope is that understanding where we have come from will prepare us to advance the cause of mosquito control in the 21st century.

That said, I am mindful of the passage from Plato's Republic that is inscribed above the entrance to the Sterling Hall of Medicine at Yale University. "Those having torches will pass them on to one another." Plato's words refer to the Greek custom that young men running a race in the Promethean Festival would carry a torch lighted at the sacrificial altar. Each runner would pass the torch to the next runner. The victorious runner was the runner whose torch remained unextinguished as he crossed the finish line. I would like to examine the lessons that can be learned from the careers of William Herms and William Reeves, and, this may surprise you, Rachel Carson, about carrying the torch of mosquito control into the future.

1. William Herms

The origins of mosquito control in California began on the east side of San Francisco Bay. In May 1904, members of the Burlingame Improvement Club sent an "urgent request" to the California Agricultural Experiment Station for help in fighting mosquitoes, which had "become exceedingly abundant and annoying." A year earlier, Mr. George Page, a member of the San Rafael Improvement Association, had contacted Charles W. Woodworth, head of the entomology section at the California Experimental Station at Berkeley, asking what could be done about mosquitoes. Woodworth suggested that the Association adopt a set of temporary measures "largely to demonstrate what could be expected in the way of control." The "temporary measures proved successful and inspired the formation of a second Improvement Club in near-by Burlingame, an exclusive summer retreat of San Francisco's wealthy elite (Quayle 1906) (Letter from H.J. Quayle, April 18, 1905 in Smith 1905).

Charles Woodworth sent a young professor named Henry Quayle to survey the situation. Quayle spent three weeks tramping through the salt marshes along the east side of San Francisco Bay. He discovered that 95% of the problem came from salt marsh mosquitoes breeding in these brackish waters. In midsummer, he delivered his report to the Club's executive committee. It was too late in the current season to take effective action. Permanent relief from the mosquito pest could be had if the Club was "earnest" and prepared to take on "the expense necessary to control this large breeding ground." If they possessed the resolve, Quayle was prepared to devise a plan (Quayle 1906). Quayle began work at Burlingame in 1905. In 1906, 10 weeks after the San Francisco Earthquake, the California Experiment Station published Quayle's *Mosquitoes of California*. Quayle, however, had left Berkeley to work at the Southern California Plant Pathology Laboratory in Whittier.

Charles Woodworth began a 2-year search for a "mosquito man" to lead the Experiment Station's anti-mosquito work. In 1908, he believed he had found his man in a thirty-two-year-old named William Herms. Born in 1876 in Portsmouth, Ohio, William Brodbeck Herms overcame substantial hurdles to become the nation's leading medical entomologist. After graduating from high school in 1894, Herms worked for four years on local farms and as an accountant, until he had saved enough money to attend nearby Baldwin-Wallace College in Berea, Ohio. During his freshman year, Herms learned of Ross's discovery of the man-malaria-mosquito cycle. "It turned," he later said, "the tide of my life." (Herms 1920).

Initially, Herms planned to become a physician. His goal was to become a medical missionary in China. By 1902 when he graduated from Baldwin-Wallace, Herms' passion for entomology eclipsed his interest in medicine. During the next six years, Herms alternated between periods of graduate study and spells of teaching in Ohio. He received fellowships from Western Reserve and Ohio State where he earned his master's degree in 1906. His desire to go to China and fight malaria remained intact. The means of achieving this end had changed. Gradually, Herms came to believe that controlling mosquitoes offered a practical solution to the malaria problem.

In the summer of 1908, Herms received a letter from C.W. Woodworth offering him a position as an Assistant Professor of Entomology at Berkeley at a salary of $1500 per year. Herms' Harvard professors advised against accepting the appointment. They "shook their heads" and told him he "was taking an awful chance." (Herms 1949) The University of California was "a dead end academically and the population of California was uncultured." Herms could do better for himself and his young wife. He should stay in Ohio or return to Cambridge and complete his doctorate. "Fortunately," William Reeves, Herms former student, observed, "Herms rationalized that California was much closer to China than Ohio and Massachusetts and he might still become a missionary by selling malaria and mosquito control in California." (Reeves 1994).

Herms retained a vivid memory of his arrival in Berkeley. It was a cold, foggy August day. His first visit to campus added to his depression. "The small rather creaky wooden building known as the Entomology Building" was a disappointment. "I almost felt," Herms later observed, "that it was all a gloomy mistake." There were no courses in the university's catalogue on medical entomology. Woodworth and Quayle's work had focused on salt marsh mosquitoes. "I must confess," Herms wrote forty years later, "that my interest in salt marsh mosquitoes was very mild— they were not vectors of malaria or of any other disease." (Herms 1949).

Six months after his arrival, Woodworth asked Herms if he would like to join the staff of the Experiment Station's Agricultural and Horticultural Demonstration Train during the spring semester of 1909. The Demonstration Train was a joint effort of the University of California and the Southern Pacific Railroad to inform the public on agricultural topics. Herms initial contribution to the exhibit consisted of "four small glass-covered boxes containing specimens of fleas, lice, flies, ticks and particularly specimens of anopheline mosquitoes, and a few charts." (Herms 1929).

Herm's experiences on the Agricultural Demonstration Train provided a basis for much of his future work. As the train made its way from southern to northern California, Herms came to appreciate California's diversity. The small farming communities in the Great Central Valley reminded him of things he had seen in the Ohio River valley. "Having been brought up in a malarial section of the country and still having the taste of quinine in my mouth," he observed, "my interest in malaria was intense." (Herms 1929). The people he encountered carried telltale signs of the wasting effect of malaria. Herms found clear evidence of malaria in the Sacramento valley and in the foothills of the Sierra Nevada Mountains. His brief remarks about medical entomology regularly elicited questions "concerning mosquitoes and malaria." He returned to Berkeley convinced that malaria constituted a significant problem in parts of California. "A determination was awakened in me," Herms later declared, "to tackle the 'job' of control." (Herms 1926-27).

In December 1909, Herms received a plaintive letter from Penryn, California. "We, here in the Placer foothill region," Fred Morgan wrote, "want to fight the malaria mosquito, but we do not know how to proceed. . .Can we not expect some aid in this. . .?" (Herms 1926-27). Residents of Placer County had long struggled against malaria. Thirty years before Morgan's letter a reporter for *The Placer Press* in near-by Auburn lamented, "almost everyone living west of Gold Hill is either down with fever, or chills and fever, or more or less affected by the miasmatic poison generated and floating around in that locality. . .What can be done to remedy the evil?" (Gray and Fontaine 1957).

In January 1910, Herms made his first visit to Penryn. A month later an anti-mosquito committee formed. With a $715 donation, Herms began the anti-mosquito campaign (Herms 1910). He believed that education was the key to winning the public's support. There was opposition. Real estate developers feared that land sales would collapse, if it became known that malaria was present in Placer County. Herms' gift as a teacher confounded his opponents. The first seasons' results were impressive. Using school attendance records, Herms estimated that his "species sanitation" methods had reduced the number of malaria cases by 50%. (Gray and Fontaine 1957).

In 1911, Herms persuaded John Guill, a newly elected Democratic assemblyman from Butte County, to introduce a bill authorizing the formation of mosquito abatement districts. Guill's bill was passed by the legislature only to be vetoed by Governor Hiram Johnson. Real estate developers feared that creation of mosquito-abatement districts would adversely affect land sales. Herms, however, persisted. Four years later, the mosquito-abatement bill was re-introduced. In the interim, Herms convinced the real estate lobby that the elimination of salt-marsh mosquitoes would benefit land sales. On May 29, 1915, Hiram Johnson signed Assembly Bill 1540 into law. Six months later, Marin County earned the distinction of becoming the Golden State's first mosquito abatement district (MAD).

Lesson 1 Mosquito Control Must Be Guided with Humility and a Sense of Service

2. William Reeves

William Reeves was born one year after the passage of the California Mosquito Abatement Act. His father was a beekeeper in Riverside. Ironically, Reeves showed little interest in bees. It was a junior high school teacher who sparked his interest in insects earning him the nickname "Billy Bugs." Reeves' interest in "bugs" became a passion while attending Riverside Junior College. He applied for summer work at the California Citrus Station. Initially, his application was turned down because he was too young. Reeves persisted. Finally, he managed an interview with the Station's director: Henry Quayle. Quayle liked the boy's persistence and hired him. Later, Reeves recalled that it was listening to the Station's entomologists talking about their work that inspired him to pursue a career in entomology (Reingold et al. 2005).

At the Citrus Station, Reeves had his first taste of research. Lunchtime conversations with Quayle and other professionals such as Harry Smith, Harold Compere, and P.H. Timberlake encouraged Reeves to pursue an undergraduate degree in entomology at Berkeley in 1936. Years later Reeves maintained that an injury playing basketball was a turning point in his academic career. A torn cartilage "was the best thing that ever happened to me, because I could no longer play basketball. . .I started studying." (Reeves 1993).

When he graduated in 1938, Reeves remained in Berkeley to pursue his doctorate. William Herms arranged a research assistantship in the entomology department and a part-time job working for Harold Gray at the Alameda County MAD. Gray assigned Reeves to answer the complaints caused by *Aedes sierrensis*. *Ae. sierrensis* is a small black mosquito with white legs that lays its eggs in tree holes. Reeves's job was to find the tree holes, climb the trees, and kill the larvae.

Curiosity led Reeves to attempt to start a colony of *Ae. sierrensis.* From Herms he learned that little was known about the insect's life history. Reeves thought that the mosquito provided a dissertation topic and at the very least knowing more about it would make his job in Alameda easier. Initially, all went well. Herms approved of the topic and Reeves set about establishing a colony. Nothing worked until Reeves discovered that the key to the *Ae. sierrensis* eggs hatching lay in the depletion of the water's oxygen level. Reeves built his dissertation around this discovery.

All seemed to go well. Reeves planned to announce his findings at the December 1940, California Mosquito Control Association (CMCA) meeting. Several months before the meeting, Herms asked Reeves to show Fred Bishopp, the chief entomologist at the USDA, his research. Bishopp had stopped in Berkeley on his way to visit the USDA's research station in Portland, Oregon. Herms request put Reeves in a difficult position. He knew that the USDA researchers were working on tree-hole mosquitoes. Reluctantly, Reeves described his discovery (Reeves 1993).

A few days before the CMCA December meeting, Harold Gray told Reeves that Claude Gjullin, one of the USDA Portland researchers, had asked for a few minutes to describe his discovery that lowering the oxygen level caused the tree-hole mosquito eggs to hatch. Herms was furious. Gjullin's presentation would cost Reeves his dissertation. Gjullin was innocent. He had known nothing about Reeves' work. What had happened was that Fred Bishopp had suggested to Gjullin that he examine the relationship of oxygen and egg hatching. Later Reeves learned that when William Herms confronted Bishopp, Bishopp had laughed saying "that's life." (Reeves 1993).

Some people have a talent for turning misfortune into opportunity. Bill Reeves was one of those individuals. Several weeks after the CMCA debacle, Reeves met with Karl J. Meyer, the director of the George Hooper Foundation in San Francisco. Reeves had heard Meyer, one of the world's leading virologists, lecture in Herm's medical entomology course on viruses. Reeves explained his situation and told Meyer he was interested in learning more about the relationship between mosquitoes and viruses. Reeves asked if it would be possible for him to work on this topic under Meyer's supervision?

Reeves meeting with Meyer came at an opportune moment. Seven hundred miles to the north in Yakima, Washington there was an encephalomyelitis outbreak. Meyer dispatched Reeves to Yakima where he joined William Hammon. Reeves and Hammon resolved the Yakima mystery of human and equine encephalomyelitis. Their mosquito pools revealed the presence of both WEE and SLE virus in *Culex tarsalis* mosquitoes. Reeves further experiments demonstrated that this species of mosquito could transmit encephalitis to laboratory animals. The discovery that *Cx. tarsalis* carried the virus in the wild was especially noteworthy. Entomologists had previously considered this mosquito as an unimportant pest. In 1943, Reeves was awarded his Ph.D. based on his research in Yakima.

Three years later, Reeves was instrumental in convincing the California Assembly to allocate $600,000 for mosquito control. The state subvention program triggered a spectacular expansion in research and scope of mosquito control in California. In 1945, there were twenty-nine MADS in California covering forty-six hundred square miles of the state. Three years later, the total area under control had expanded to more than fifteen thousand square miles, while the number of MADs had increased to forty-two. By 1954 there were fifty-three agencies covering thirty-thousand square miles. William Reeves deserves a sizeable portion of the credit for this dramatic increase (Gray 1949).

"Billy Bugs" Reeves contributions to understanding mosquitoes had a transformative effect on mosquito control. Reeves invented the term "arborvirus" and pioneered the use of sentinel chickens and florescent dyes in tracking mosquito migrations. Those that encountered Reeves knew him to be a hard taskmaster. Reeves was a consummate teacher and researcher. He demanded much from himself and expected no less from others. Once during his tenure as Dean of the School of Public Health at Berkeley he visited his colleague, Leonard Symes. The two men sat in Symes backyard talking. Symes eight-year-old son, David, sat listening to the two men. Reeves looked at the boy and asked, "do you know what my job is?" The boy responded, "you are a psychologist who is interested in mosquitoes." This entomologist would-be psychologist's career provides a blueprint for a life devoted to science and serving the public (Pincock 2004).

Lesson 2: Mosquito Control Must Be Guided by Science

3. Rachel Carson

Rachel Carson was born in 1907 in Springdale, Pennsylvania. By her own account, it was a pristine environment that was spoiled by the presence of two coal fired power plants. As a child, her mother encouraged her interest in nature. Carson attended Pennsylvania College for Women (later, Chatham College). She intended to major in English but an encounter with a brilliant, talented, woman zoology professor led her to change her major to biology.

Carson graduated from college at the beginning of the Great Depression. She found work on a short research project at Woods Hole in the summer of 1929 before beginning graduate studies at Johns Hopkins in zoology. When Carson completed her masters degree she "had a degree but no prospect for full-time employment." (Lear 2009). Carson struggled to support herself as a writer. Her initial efforts earned her a series of rejection slips. Carson persisted and Elmer Higgins at the U.S. Bureau of Fisheries hired her to write the scripts for "seven-minute fish tales" for radio broadcasts. The radio programs led to Carson's being hired to contribute articles to the *Baltimore Sun.*

Carson's talent won her a full-time position as an aquatic biologist at the Bureau of Fisheries in Washington, D.C. in 1936. Three years later the Bureau of Fisheries was reorganized becoming the U.S. Fish and Wildlife Service (FWS). Carson published her first book, *Under the Sea Wind*, in 1941. In 1949, she became the chief officer in charge of all of the FWS's publications. Two years later, she published *The Sea Around Us* which became a Book of the Month Club selection.

Carson had long been interested in "the role of poisons in the environment." In 1945, she submitted a proposal for an article about DDT to the *Reader's Digest.* The turning point for Carson, however, came a decade later when she received the copy of a letter published in the *Boston Herald*. The letter decried the alarming decline in bird numbers at a New Hampshire bird sanctuary which had recently been sprayed with DDT. Olga Owens Huckins, the former literary editor of the *Boston Post,* forwarded a copy of a letter about a property which "had been sprayed repeatedly for mosquitoes in the summer of 1957." (Lear 2009). Carson began work. Four years later, in June 1962, *The New Yorker Magazine* published the first of three installments of *Silent Spring.* The book appeared in September. The reaction was immediate. Carson was attacked as an alarmist. "The major claims of Rachel Carson's book, *Silent Spring,*" Dr. Robert White-Stevens, spokesman for the chemical industry declared, "are gross distortions of actual facts, completely unsupported by scientific, experimental evidence, and general practical experience in the field. Her suggestion that pesticides are in fact biocides destroying all life is obviously absurd." (Leonard 1964).

Perhaps the most revealing response came in a letter to the *New Yorker.* An unnamed Californian wrote:

> Miss Rachel Carson's reference to the selfishness of the insecticide manufacturers probably reflects her Communist sympathies, like a lot of our writers these days. We can live without birds and animals, but, as the current market slump shows, we cannot live without business. As for the insects, isn't it just like a woman to be scared to death of a few little bugs! As long as we have the H-Bomb everything will be O.K. (1995).

Carson's jeremiad came at the moment of growing concern about threats to public health. In 1959, three weeks before Thanksgiving, Arthur S. Flemming, the Secretary of Health, Education and Welfare, advised Americans to exercise caution in eating cranberries because "a small portion of the crop from the Pacific Northwest had recently tested positive for the herbicide." (Tortorello 2015) Two years later, a drug that was widely used in Europe, thalidomide, to treat nausea in pregnant women, was shown to produce severe birth defects. Carson's *Silent Spring* arrived in bookstores in this context. Americans faith in technological wonders was called into question. There were mistakes in *Silent Spring*. There were overstatements. The truth of Carson's fundamental message, however, was clear: We are all in this together.

Carson's *Silent Spring* deserves our attention today just as it did when it was first published. Her position on the use of insecticides is nuanced. Early in the book she observes:

"It is not my contention that chemical insecticides must never be used. I do contend that we have put poisons and biologically potent chemicals indiscriminately into the hands of persons largely, or wholly ignorant of their potentials for harm." (Carson 1962). A few weeks before her death in 1964, Carson answered her critics in a short article in the Audubon Magazine. "We must have insect control," she wrote. "I do not favor turning nature over to the insects. I favor the sparing, selective, intelligent use of chemicals. It is the indiscriminate blanket spraying which I oppose. . . We do not ask that all chemicals be abandoned. . . We ask for moderation." (Beyl 1992).

"Moderation and Intelligence:" These are, I believe, the watchwords of this quiet woman who had the temerity to challenge her contemporaries.

Lesson 3: The responsibility of Science and Scientists to Protecting the Environment

Conclusions

William Herms, William Reeves and Rachel Carson advanced the torch of mosquito control. They ran a good race. Their message was a call for science, service to the public, and protection of the environment. This was the message that Maurice Provost articulated seven years before the publication of *Silent Spring* in his presidential address to the Florida Anti-Mosquito Association. In 1961, a year before the publication of *Silent Spring*, Provost told the members of the Vero Garden Club:

> The day is gone when we can improve our relations with our fellow men and fellow animals by simply reaching for a gun. We need to understand, and for this we must learn, and so we need only learn, learn, and learn some more to appreciate that our natural environment is full of wonder and beauty — even if we learn about such a practical phenomenon as the common milieu of birds, mosquitoes, and viruses. Inquiring into our living environment is the surest way of enhancing our awe towards it and our reverence for it (Provost 1961).

If we are to carry the torch of mosquito control forward, we must not forget the message of individuals like William Herms, "Billy Bugs" Reeves, and, yes, Rachel Carson. Mosquito control workers must do their work with humility and awe as scientists, public health workers, and ecologists.

This was precisely the message that William Herms delivered in his final presentation to the Californian Mosquito Control Association in January 1949.

> Yours is, therefore, a serious responsibility based on *goodwill*. Your constituents look expectantly and confidently to you for the abatement of annoying and disease-bearing mosquitoes. You have a job to do — do it with sincerity and devotion. Continue to be worthy of the good will of your constituents. (Herms 1949).

Sound advice for those carrying the torch of mosquito control in the twenty-first century.

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A Road Not Travelled – Re-thinking malaria control in Africa through mosquito control

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Abstract

The knowledge of mosquito control pioneered by our forefathers at the turn of the 19th century brought forth the ingenuity of scientists and physicians for controlling malaria. The mosquito control practitioners in the United States championed the enactment of mosquito abatement laws that eventually helped turn the tide against mosquito-borne diseases. In 1915, the Rockefeller Foundation entered the effort targeting malaria control in the United States and around the globe, by applying innovations from mosquito control practitioners in the USA to the rest of the world. As mosquito abatement districts and their work came of age in the 1920s and 1930s, their research pursuits were replicated in newly built international research facilities funded through the Rockefeller Foundation. The research pioneered in such research institutions and other non-profit entities in Africa was stellar; however, the operational application that practically lowers disease incidence was never followed. My presentation describes the formation of the World Health Organization and its role in the trajectory of malaria control in Africa. It advances the re-thinking of malaria control by engaging local scientists and emphasizing building infrastructure onto which interventions against malaria should be conducted.

Background

After Sir Ronald Ross demonstrated in 1899 that mosquitoes transmit malaria, malariologists and public health enthusiasts started the quest to control this disease worldwide. The effort intensified in the United States, Europe, Southeast Asia, India, and other places, at breathtaking speed. In 1900, Dr Walter Reed of the US Army and the Reed Commission demonstrated that mosquitoes transmitted yellow fever and a year later implemented the most ambitious yellow fever control effort ever by controlling *Aedes aegypti* in Havana, Cuba. Similar efforts by Fred Soper funded by Rockefeller Foundation eliminated this species and urban yellow fever from most parts of South America.

Prof. Charles Woodworth of the University of California, Berkeley, and Prof. John B. Smith of Rutgers University in New Jersey pioneered mosquito control in the US, where they helped public health and mosquito control practitioners first focus control of the mosquito and then the pathogen. In 1906, Prof Smith was instrumental in rallying local communities to organize the first mosquito control program in the United States. Prof William Herms was recruited by UC Berkeley to lead the effort of controlling malaria in California; however, a few years into this position he switched his focus from malaria to mosquito control, championing the enactment of mosquito abatement laws in the state that eventually were passed in 1915 (Gray and Fountaine 1957; Patterson 2009). Similar efforts by equally engaged practitioners such as Don Rees in Utah, John Mulrennan and Maurice Provost in Florida, and others expanded the enactment of mosquito abatement laws throughout the USA (Hughes, 1993).

The practice of mosquito control as the major intervention method for the control of malaria and other mosquito-borne diseases expanded to other parts of the world through the efforts of government and many non-governmental entities such as the Rockefeller Foundation of New York, Welcome Trust of Great Britain, Pasteur Institute of France, Japan International Cooperation Agency, and many others. These entities helped to expand the capacity for research on malaria and other tropical diseases on the African Continent. The Rockefeller Foundation's International Board of Health was instrumental in this expansion, building research institutes and field laboratories in many African cities (Stapleton, 2004) such as Johannesburg, Cairo, Ibadan, Amani and Ifakara Tanzania, Entebbe and Kabale in Uganda, and Muguga in Kenya. Institutions included the Entebbe Virus Research Institute and the Kabale Virus Research Center in Uganda, Ifakara Research Institute in Tanzania, Kenya Medical Research Institute in Kenya and others that became part of the East Africa Medical Research Institute. It was in these facilities from the 1930s through the 1950s that many mosquito-transmitted viruses such as West Nile, Rift Valley Fever, chikungunya, O'nyong Nyong and others were first described (Stapleton 2004).

This expansion built strong research institutions; today Africa has great training facilities and has been conducting important research. However, the expansion of basic research in Africa proceeded without similar growth in applied research on mosquito control, the operational approach known to achieve tangible results in reducing mosquitoes-borne disease world over. Operational work focusing on mosquito control has been the main tool for controlling malaria, reducing this disease in many parts of the world to the point of eradication or to a point where it is no longer a major source of morbidity and mortality. What is not clear is why similar operational efforts have not been undertaken at a large scale in many parts of Africa?

Formation of the World Health Organization: At the end of World War II the United Nations commissioned the Interim Commission on Health in 1946, a time when new chemistries for mosquito control and remedies for treating malaria were ushered onto the market. The Commission adopted some policies of the Rockefeller Foundation's International Board of Health, including malaria eradication. On 7 Apr 1948, the United Nations chartered the World Health Organization with the eradication of malaria as its official policy (WHO, 1958). Funding from the Rockefeller Foundation and several other global organizations and institutions under WHO guidance helped establish and provide a pipeline for excellent investigators to conduct research on the African Continent, including Drs. M. T. Gilles, J. D. Gillies, A. J. Haddow, P. F Mattingly, W. H. Lumsden, C. Teasdale, A. McClelland, G. B. White, M. Colluzzi, M. Service and others, some of which are still doing great work on the continent (Boyd 1949, Gilles et al. 1993).

African scientists and malaria control professionals past and present have had great experience in basic research, but hardly know the best practices of traditional mosquito control used world over to reduce mosquito-borne diseases, especially using larval source management through an integrated vector management strategy. Until recently, WHO did not recommend larval mosquito control as part of a malaria control strategy in Africa. In fact, mosquito larvicides used elsewhere in the world are not available in many African countries, because their availability must be approved by the WHO Pesticide Evaluation Scheme (aka WHOPES). Many pesticide manufacturers around the world gave up in frustration second availing their products to African countries because of, first, the lack of support and the restricted approval regimen by WHO, and second, larval source management was not permitted as an intervention against malaria until about 2013. The latter was later recommended in limited circumstances as a supplement to malaria control programs in African countries (WHO 2013, Mir Mulla, 2015, Pers. Commun., Major Dhillon, 2023, Pers Commun.). Many of the African malaria control practitioners need training to help them include larval mosquito control among the malaria control strategies.

Outcome of the Malaria Eradication Effort: The malaria eradication plan adopted by the World Health Organization at its inception focused on mosquito control and treating malaria patients. This two-pronged approach aimed at reducing malaria cases by controlling infected female mosquitoes by applying massive quantities of pesticides [initially DDT indoor spray] and treating infected people [initially with chloroquine]. This approach was conducted at differing levels and was supported by regional health organizations in Europe, Middle East, Asia, Southeast Asia, and the Americas. With the exception of Cameroun and Liberia, the eradication program was not initiated in Africa due to the reasons explained in the 10th Anniversary Report of the World Health Organization (WHO, 1958). Although many countries worldwide retreated thereafter from eradication efforts, malaria cases never returned to the pre-eradication era level. For Africa the statistics has remained tragically high then to present for example, in 1958, there were 300 million cases of malaria and 3 million deaths worldwide, whereas in 2022, there were 246 million cases of malaria with 618,000 deaths, with 95% of these cases and deaths occurring on the African continent (WHO, 1958; 2022).

Despite the failure to launch the eradication program in Africa, mosquito control as a malaria intervention was implemented in many settler communities in several African countries before independence, and these efforts persisted through several decades after independence. These countries included Kenya, Uganda, Tanzania, Zambia, Nigeria, Ghana, Zimbabwe, South Africa and several others (W. L. Kilama, 1993, Pers. Commun.; J-P., Mutebi, 2024, Pers Commun.). If the control efforts in settler communities were expanded or sustained by adopting policies in 1950s through the 1980s, the malaria outcomes would most likely have been different today.

The WHO Policy Stagnation and Incremental Re-Engagement: Since the 10th Anniversary of WHO in 1958, the malaria control policy in all 54 countries in Africa has relied on the WHO direction determined by colonial governments that transitioned into the independent governments of today. The WHO representative office headed by the Program Director in each of these countries approves national malaria control policies. Over several decades, the policies have shifted away from mosquito control to case detection and treatment as the main control strategy. This strategy failed to impact the number of malaria cases until the introduction of impregnated bed nets and indoor insecticide treatments were approved (Curtis, 1990; Glaser, 2009).

A policy focusing on the detection and treatment of the malaria pathogen is not the way vector-borne diseases have been successfully managed; controlling the mosquito vector is the best strategy. In Africa, the policy from the 1960s through the 1980s that solely focused on controlling the pathogen failed as malaria resistance to chloroquine, then mefloquine, sulfadoxine, as well as many other treatments increased over the years. This strategy gave the physicians and bureaucrats the lead in malaria control instead of empowering mosquito control professionals. The approach to controlling malaria focused on mosquito control gives the mosquito control professionals and ordinary folks in the community a significant role in intervention efforts. This approach followed practices used in areas where malaria has been controlled. The WHO policy on malaria control has been limited to a few malaria intervention strategies contrary to the broad expert knowledge required to achieve control. WHO has provided significant funding to these programs.

The late 1980s, the pioneering work of British researchers such as Prof Chris Curtis on the use of bed nets, and there after permethrin impregnated bed nets, was advanced as the primary intervention for malaria control that focused on protecting people from mosquito bites. In the 1990s, WHO adopted the use of impregnated bed nets as a secondary intervention in addition to indoor insecticide residual spray treatments (Curtis, 1990). These remain the only mosquito control interventions recommended by WHO today, with larval control considered only as a supplement (WHO 2013). Allowing African professional folks to train and re-think malaria control through mosquito control may help re-orient them to understand some of available alternative methods such as larval source management implemented in a comprehensive integrated vector management program.

Currently, Africa has some of the best-trained people in medical entomology and parasitology to enlist in the fight against malaria. The main handicap is that most of those trained have their expertise in laboratory research and not field in operational control work. They are quite knowledgeable in conducting bioassays for pesticide resistance, but limited in applying pesticides to control larval mosquitoes in a comprehensive integrated vector management (IVM) strategy. The future of malaria control in Africa may require an approach that incorporates IVM as a strategy and focuses on mosquito control. Whether or not the structure for mosquito abatement districts takes hold, a focus shifting to mosquito control as a strategy will be necessary for success.

Re-think Malaria Control Through Expanded Mosquito Control Programs: Malaria control in Africa should be refocused on the vector instead of the pathogen as argued above. Such a refocus will relegate physicians, who have been at the forefront of malaria control on the continent since the demise of the eradication effort, to a secondary position and bring mosquito control professionals to the forefront. Through their leadership, it will be easier to engage local communities and enable governments to build infrastructure that may implement comprehensive IVM into malaria control (Dickson et al. 2022).

Training staff on the broad knowledge of IVM that incorporates all aspects of larval and adult mosquito control, and implementing such programs across malaria endemic areas is important. Such IVM includes the current interventions of bed nets and indoor residual insecticide spaying. Africa has a large, young cadre of scientists well educated in bench and laboratory work rather than operational work. Mosquito control requires extensive field work to understand the biology and ecology of vector mosquitoes. The exposure of African scientists and other practitioners to the mosquito abatement approach will go a long way in equipping these scientists with the right tools for moving malaria control to the next level.

The African professionals in the field with help of the Society of Vector Ecologists, the American Mosquito Control Association and the American Society of Tropical Medicine and Hygiene helped to organize an association known as the Pan African Mosquito Control Association. The association is about 10 years old and has brought together scientists, government agencies, practitioners, and donors from inside and outside Africa to help chart the best way forward for reducing malaria cases in the future. The African scientists and practitioners are uniquely positioned to guide and implement a proper IVM in their malaria control programs; however, they are handicapped because of lack of knowledge of the practice of controlling mosquitoes including Larval Source Management (Ochomo et al. 2024).

The AMCA-PAMCA Memorandum of Understanding: In 2022, the American Mosquito Control Association (AMCA) entered into a Memorandum of Understanding (MOU) with the Pan African Mosquito Control Association to provide training for African scientists and technicians in mosquito abatement-style mosquito control operations. The AMCA has encouraged individual and regional membership to support the initiative. Such training will help trainees acquire knowledge and best practices of mosquito control in a mosquito abatement style setting that they can bring back to their countries to assist in developing programs that incorporate mosquito control techniques that have routinely been used to control mosquito-borne diseases in California and the rest of the United States. Participating districts have included the Salt Lake City Mosquito Abatement District in Utah, North Shore Mosquito Abatement District in Illinois, St Augustine Mosquito Abatement District in Florida, and many others (Ochomo et al, 2023).

The above MOU has already enabled several African scientists and operations staff to visit local mosquito abatement districts in California. With Sacramento-Yolo Mosquito and Vector Control District has taken a leading role by hosting two vector control members from PAMCA at their facility each of the last three years (2022, 2023, and 2024). The Mosquito Control and Vector Association of California as an affiliate member of AMCA created an International Fund into which member agencies of the Association may donate funds directly to support African trainees attend programs at local mosquito control districts. In addition, funds can be disbursed through application to the Association's president to support staff from member agencies at the invitation of PAMCA to travel to African countries to help in implementing mosquito control programs within their jurisdiction.

Conclusion

Africans and friends of Africa working in the mosquito abatement industry are urged to assist in developing programs that use integrated vector management principles and elevate mosquito control on the African continent and to help increase the networks and capacity for such operations. Many colleagues across the United States are committed to this endeavor, to help shape the next chapter of malaria control in Africa with the goal of achieving zero malaria cases over the next several decades. We are all aware of many research initiatives, especially innovative technologies including irradiated sterile male release, *Wolbachia*-infected sterile male releases, genetically modified male mosquito releases, and malaria refractory mosquito releases that are ongoing. We believe developing the infrastructure for mosquito control as advocated in this article will be instrumental in the implementation of such innovative technologies once they are ready for deployment.

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Dengue and chikungunya virus loads in the mosquito *Aedes aegypti* are determined by distinct genetic architectures

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Introduction

Aedes aegypti, the primary vector for arboviruses such as dengue (DENV) and chikungunya (CHIKV), displays distinct interactions with both viruses. Chikungunya virus exhibits a more rapid traversal of the mosquito body and elicits fewer conventional antiviral responses compared to DENV. With the expanding global reach of CHIKV, it is crucial to gain insights into the mosquito's innate responses to CHIKV, enabling comparisons of the vector's coevolutionary history with DENV and pinpointing potential genetic modification targets within natural mosquito populations.

Methods

In our previously published study (Novelo *et al.*, 2023), we employed a modified full-sibling design to assess the impact of mosquito genetic diversity on viral loads of both DENV and CHIKV. Briefly, six to seven-day old virgin adult *Aedes aegypti* females were blood-fed and then 250 virgin males were added to the cage and allowed to mate. Gravid females were placed in small individual housings containing moist filter paper. Egg papers were collected and dried 3–4 days later for short-term storage. The eggs of each family were hatched separately, and after pupation, females were separated and split into two cups with a minimum of eight individuals per cup. These females were maintained on sucrose until vector competence experiments. Prior to infections, mosquitoes were starved for 24 h. Half of the 6–7-day old females of each family were challenged with DENV-2 (1st cup) and the other half (2nd cup) with CHIKV at equal viral titers using a 1:1 mix of the frozen titrated aliquots and human blood. Seven days post-infection, whole mosquitoes were collected, homogenized, and stored in TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) at −80°C. RNA was extracted according to the manufacturer's protocol, and from this RNA, DENV and CHIKV viral loads were quantified.

Results and Discussion

Our findings showed significant differences in heritability for DENV (40%) and CHIKV (18%) viral loads. Intriguingly, we observed no genetic correlation between DENV and CHIKV loads between siblings of the same families, indicating that *Ae. aegypti* employs distinct genetic mechanisms when responding to these two viruses. Furthermore, we conducted a comprehensive analysis of genome-wide gene expression patterns in the High and Low CHIKV viral load families, representing the extremes viral susceptibility phenotypes. Through RNA sequencing, we consistently identified only two genetic loci distinguishing the High and Low families. One locus corresponded to a long non-coding RNA previously identified in mosquito screens post-infection, whereas the other belongs to the Salivary Gland Specific (SGS) gene family. Interestingly, this latter gene is associated with horizontal gene transfer events between mosquitoes and the endosymbiotic bacterium *Wolbachia*. our study marks the first connection between the SGS gene and a mosquito phenotype. Exploring the molecular intricacies of how this gene contributes to viral control in mosquitoes may offer insights into its role in the context of *Wolbachia* as well. Our study allowed us to understand the genetic basis of the mosquito's interaction with these arboviruses not only enhancing our knowledge of vector-virus dynamics but also holding promise for the development of innovative control strategies. Targeting specific genetic factors that influence viral load and transmission in mosquitoes could pave the way for the development of novel genetic modification approaches, such as gene editing or gene drive technologies.

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The impact of aromatic plants on BG-Sentinel II trap efficacy

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Abstract

Aromatic plants such as lavender (*Lavandula* sp.) and geranium (*Geranium* sp.) are frequently found in residential front yards. Their prevalence can be attributed to their remarkable drought tolerance, a highly sought-after trait in the arid Mediterranean climate of Fresno County, California. These aromatic plants are known to emit volatile compounds when agitated. This study determined whether aromatic plants in residential landscaping deter mosquitoes, resulting in decreased mosquito activity on the property.

The study was conducted in Clovis, California using BG-Sentinel (II) traps to sample the mosquito population. Traps were set weekly next to lavender, geranium, or a non-aromatic control plant. There was no significant difference in the number of mosquitoes collected adjacent to aromatic plants when compared to the control group of non-aromatic plants.

Although there were slightly more mosquitoes collected overall at the control sites, this difference was not statistically significant.

Evaluating the vector potential of the ln2Care mosquito traps against the invasive yellow Fever Mosquito, *Aedes aegypti* (L.) in Visalia, California

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Abstract

Aedes aegypti (L.) mosquito populations are very difficult to control due to their container-breeding behavior and resistance to chemical insecticides. Autodissemination is a method of mosquito control that uses the ability of egg-laying mosquito females to find containers and contaminate them with the insect growth regulators such as pyriproxyfen (PPF) to kill their offspring. As the *Ae. aegypti* females visit multiple oviposition sites during "skip oviposition", PPF is deposited in multiple oviposition sites where it affects larval and pupal development by preventing the emergence of adult mosquitoes. We conducted this 2023 preliminary study in 25 volunteer properties in northern Visalia, California where abundant *Ae. aegypti* populations were found between July 12 and October 31, 2023, to demonstrate that the use of ln2Care Mosquito Traps can be a novel tool used to reduce *Ae. aegypti* mosquitoes within our District. Field results showed that after the ln2Care Mosquito Traps were used, treatment site ovitraps had more dead larvae and pupae than the control site ovitraps, confirming that the autodissemination feature of the combination of PPF with the entomopathogenic fungus, *Beavaria bassiana,* in the ln2Care Mosquito Traps was achieved.

Optimizing the synchronization of *Aedes aegypti* larval development

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Abstract

Aedes aegypti, a potential vector of dengue, chikungunya and Zika viruses, is rapidly spreading in California and mosquito control agencies are urgently seeking innovative solutions. The Sterile Insect Technique (SIT) is emerging as a biological control tool for invasive mosquitoes. This study aimed to synchronize *Ae. aegypti* larval development and adult emergence in the laboratory conditions. Two and four month old *Ae. aegypti* eggs were hatched at different densities (500, 750, 1,000 eggs/flask) in triplicates. Hatching success, larval development and male emergence was compared between the egg ages and densities. Hatching younger eggs at lower densities provided a high hatching success (95%), well synchronized larval development and a synchronous (93%) male emergence in the first two days of adult emergence. Conversely, higher egg density led to lower hatching success, longer larval development, and delayed adult emergence. Our findings highlight how to optimize larval synchronization of mosquito colonies to efficiently rear mosquitoes for SIT purposes in limited lab spaces and collect the majority of male mosquitoes in the first days of emergence.

Effect of temperature on *Wolbachia* during *Cx pipiens* development

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Abstract

During the outbreak of West Nile Virus (WNV) in the United States in 2003, *Cx. pipiens* complex was estimated to contribute to approximately 80% of the human cases. This could be related to the ubiquitous presence of the mosquito, its feeding on birds which are maintenance hosdts of the virus, its high local abundance, and its close association with domestic environments. *Culex pipiens* is one of the main WNV vectors in North America and is well known for its natural infection with *Wolbachia*. When mating occurs between insects of the same species with different *Wolbachia* infection status, Cytoplasmic Incompatibility (*CI*) and embryonic mortality can result. Temperature can influence the mosquito life cycle and *Wolbachia* replication and density within its host. This research aims to determine the effect of temperature on *Wolbachia* presence and *Cx. pipiens* development. DNA molecular analysis and microscopy techniques (FISH) were used for *Wolbachia* detection and relative quantification in embryo tissues from *Cx. pipiens* reared at three different temperatures. The preliminary outcome included an image analysis of *Wolbachia*'s location and differences in its relative abundance when the mosquito eggs are submitted to temperature treatments throughout development. At higher temperatures, *Wolbachia* abundance is likely to be reduced which could mean greater susceptibility to WNV in the subsequent generations of the *Cx. pipiens* complex.

Minimum temperature modulates *Aedes aegypti* population dynamics in the West Valley region of San Bernardino County, California

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Abstract

Aedes aegypti has spread throughout most of southern California in recent years. We analyzed the effect of weather factors on the population dynamics of *Ae. aegypti* in the West Valley region of San Bernardino County. Weather data obtained from 2016 to 2023 from local meteorological stations included weekly average maximum and minimum temperature, average temperature, precipitation, and wind speed. Corresponding weekly mosquito count data that were collected using BG Sentinel traps were utilized for this analysis. Linear regression models were applied to assess the temporal relationships between weather factors and mosquito counts. Among weather factors, weekly average minimum temperature was positively correlated ($r=0.86$; $P<0.001$) with weekly *Ae aegypti* counts. The regression model showed that minimum temperature explained 53% of the weekly variability in mosquito counts. The impact of precipitation was positive when lagged by two weeks ($r=0.62$; $P<0.05$). Overall, our analysis indicated that minimum temperature modulates *Ae. aegypti* population dynamics in the West Valley region of San Bernardino County.

Knowledge, attitudes, and perceptions of DEET-based mosquito repellent in Placer County, California

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Abstract

Mosquito-borne infectious diseases (MBIDs) are substantial contributors towards the global infectious disease burden, therefore necessitating proper prevention and control strategies. Due to the lack of adequate vaccines and long-term mosquito control, the adoption of personal protective behaviors (PPBs) is vital to reduce transmission. Consequently, understanding the processes underlying the use of PPBs is essential. Our study examined the relationships among knowledge, attitudes, and practices in the context of the use of DEET-based mosquito repellent, a prominent PPB. Study participants were recruited across two community events in Placer County, California, and data was collected through two knowledge, attitudes, and practices (KAP) surveys on Google Forms over a three-week period. Results indicated that (1) exposure perceptions, wariness risk attitude, and social attitudes for mosquito repellent use predicted mosquito repellent use; (2) wariness risk attitude and social attitudes for mosquito repellent use predicted ownership of mosquito repellent; and (3) knowledge predicted personal attitudes for or against mosquito repellent use. These findings provided valuable insight to inform future public health interventions in the sphere of mosquito control and prevention.

Proper exclusion techniques in rodent abatement

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Abstract

In Alameda County, one of the main service calls we respond to are for rodents inside structures. Commensal rodents including the roof rat (*Rattus rattus*), Norway rat (*Rattus norvegicus*), and house mouse (*Mus musculus*) are common and pose a public health risk when living alongside humans. Although eradicating rodents from the outdoor environment is often not feasible, it is usually possible to eliminate rodents from the interior of buildings. Although trapping can be an important step to eliminate an existing population, the most important aspect of preventing future rodent infestations in a structure is proper exclusion. In our inspections it is often apparent that proper exclusion techniques are not known or have not been implemented. Many inadequate repairs are both unsightly and do not stop rodents from entering structures. We discussed examples of improper repairs and how to fix them in a permanent and aesthetically pleasing manner.

Cup bioassay design and cost considerations for use in evaluating WALS Treatments

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Abstract

Stagnant water sources are abundant in many residential backyards and often become cryptic mosquito sources that are difficult to access and abate. These cryptic sources allow container- mosquitoes such as *Aedes aegypti* to flourish and create public nuisances for residents. Because of the difficulty in accessing backyard sources, staff at Northwest Mosquito and Vector Control District implemented Wide-Area Larvicide Sprays, or WALS, throughout specific neighborhoods in Riverside, CA in late summer and fall of 2023 based on mosquito surveillance data and resident complaints. We used cup bioassays to determine if the pesticide spray reached the target area at a functional dosage to effectively kill mosquito larvae. There also were cost considerations when designing the bioassay. We used disposable cups with matching lids taped to disposable plates as anchors. The lids were important to protect the inside of the bioassay cups from cross-contamination when the cups were transported back to the laboratory. Future designs will continue to incorporate easily available, inexpensive materials that allow reliable data collection. Evaluating the cost considerations of each component of an IVM program can lead to programmatic improvements in both effectiveness and sustainability.

Yellowjacket nest distribution in Alameda County

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Abstract

Yellowjackets are a common pest in the summer months within Alameda County. Alameda County Vector Control Services District responds to requests for services from the public to identify and treat or remove yellowjackets' nests. In Alameda County, the months with the greatest number of requests for services regarding yellow jacket activity typically are June – October. From 2016 to 2022, there has been an increasing trend in the number of requests for service each year. Within this period, the number of requests has varied markedly; for example, in 2016 there were 167 requests for service, while in 2017 there wer 338, in 2018 there were 219 and in 2019 there were 420. This alternating pattern has continued through 2023. We also summaried data regarding the distribution of yellowjacket activity throughout the county, which may have been affected by weather, elevation, rainfall, or other environmental factors. Cities in the northern county, specifically Oakland and Berkeley, were shown to have the highest percentage of the requests for service regarding yellowjackets. Analyzing the spatial distribution pattern of requests as well as annual changes in which months consistently have the highest number of requests and how this pattern alternates from year to year is important to prepare our biologists to successfully assist the residents of the county.

Sewers and Norway Rats in the City of Oakland

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Abstract

Norway rats are a significant public health problem that are commonly associated with urban sewer systems, which serve as a population reservoir. Sewer baiting using rodenticide bait is a common intervention for the control of Norway rats in urban sewers. However, studies on the effectiveness of sewer baiting for controlling Norway rats are sparse, and there are very few studies on Norway rat ecology. Although sewer baiting has been practiced for decades, there has been little research investigating the relationship between below-ground Norway rat activity and above-ground contact between humans and Norway rats.

The Alameda County Vector Control Services District (ACVCSD) was established in 1984 by the Alameda County Board of Supervisors to provide comprehensive vector control services to residents of the county. Vector Control Biologists (VCBs) at ACVCSD respond to requests for service (RFS) made by residents of Alameda County by providing inspections and recommendations for reported vector-related issues. RFS data, including location, date of request, and vector species, is recorded in ACVCSD's internal database. Alameda County's largest city, the City of Oakland, accounts for nearly half of all rat-related RFSs during the years 2018–2022.

Sanitary sewer infrastructure in Oakland, some of which is over 100 years old, can develop cracks that allow Norway rats to burrow from the sewers to the surface. In 1987, the City of Oakland recognized that it had a severe rodent problem as a result of its sewer infrastructure and voted to provide additional funding to control rodents in its sewers. The resulting contract between ACVCSD and the City of Oakland involves the inspection of 8,000 manholes per year and treatment of those manholes that have Norway rat activity. ACVCSD also receives RFS from residents of Alameda County for various vectors, including urban rodents.

Using the data generated from ACVCSD's sewer baiting program and RFS records during the years 2018–2022, we found a significant correlation between proximity to active Norway rat populations in the sewer and above-ground Norway rat contact with Oakland residents ($\chi^2 = 124.9$, df = 1, $p < 2.2\text{e-}16$). In addition, rat RFSs located $\leq$ 200m from an active sewer population were found to have 2.06 times the odds of having Norway rat activity compared to those that were further distant (OR: 2.06, 99% CI: 1.75–2.44). These findings highlight the importance of sewer-based interventions as part of an IPM strategy for controlling Norway rats.

Collaborating with Cities to Manage Problematic Stormwater Sources in the Coachella Valley

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Abstract

Mosquito control in urban areas is fundamental for protecting public health and enhancing the quality of life for residents. How we achieve mosquito control in the urban environment and bring relief to the residents of the Coachella Valley is carried out by surveillance of know breeding sources, pesticide applications, and source reduction where conventional methods do not work. Habitat modification and regulatory measures are integrated into Best Management Practices (BMPs) to create mosquito-resistant urban landscapes. Collaboration and partnerships with local government agencies and community groups are important to implement mosquito control. Regular evaluation and review of control measures helps to design strategies for local conditions and mosquito species, ensuring effective and environmentally responsible mosquito control practices. BMPs serve as a valuable framework for urban mosquito control programs, prioritizing public health. Our project focuses on *Culex quinquefasciatus* and its association with urban sources in the Coachella Valley. Collaboration between the city works departments and our agency expanded our understanding of urban drainage infrastructure and function, facilitating mosquito control.

Introduction

The Coachella Valley Mosquito and Vector Control District (District) is dedicated to enhancing the quality of life for our community by providing effective and environmentally sound vector control and vector-borne disease prevention programs. To achieve this, the Operations Department established an approach to mitigate mosquito larval sources in urban areas throughout the Coachella Valley. The strategy connected district staff with the city public works agencies to collaborate, develop, and carry out solutions that supported the district's mission and supplemented the Operations Department' s conventional control methods. The outcomes of this collaboration became what we defined as the Best Management Practices (BMPs). The idea behind these BMP started when one of our board members, Clive Weightman, asked if there was anything he could do to bring the operations department together to form a partnership with the city of Indian Wells.

Objectives

The development and implementation of BMPs had many goals, including learning which cities had sources that were contributing to mosquito production, the types of mosquito sources present in the urban environment, why these sources continued to contribute to mosquito production and how could we develop a collaborative campaign with city agencies. The District's jurisdiction spans the entire Coachella Valley. Every major and minor city within its boundary has sources that contribute mosquito production. Of the major cities, Coachella, Indio, La Quinta, Indian Wells, Palm Desert, Rancho Mirage, Cathedral City, Palm Springs, and Desert Hot Springs were contacted for support. From our field inspections we identified that catch basins, dry wells, siphon drains, yard drains, drainage gutters, and retention basins were the sources in the urban environment that were producing mosquitoes. These types of stormwater sources became suitable habitats because they stopped functioning as intended due to an aging infrastructure, poor design, or the drainage systems had become overloaded with leaf litter, sand, and trash. When the district approached the city agencies our intention was to seek long and short-term solutions that would mitigate these sites as mosquito sources.

Methods

Several steps were taken to carry out the BMPs and collaborate with city agencies to make a difference in the community. The work was conducted by a Lead Vector Control Technician (VCT III). Initially the VCT III gathered data on sites that were frequently treated and/or visited by Vector Control Technicians I and II. Data was reviewed through our proprietary software called the "Ops application", that resembles ESRI's mobile system. A list of sites was generated per city to be investigated in the field. A Lead Vector Control Technician reviewed these sites to discover why these were difficult to manage. During the review process pictures were taken and notes written describing the conditions observed. Data recorded included: Is the site holding water? Why is the site holding water? Where is the water coming from? What is

Figure 1.—Example of a catch basin clean out in Palm Springs. A) Veolia North America, a contracted group, cleaning out a catch basin in the city of Palm Springs using a GapVax truck. Water is used in conjunction with the built-in vacuum to clean out the drains. B) Catch basin site before cleaning. C) Catch basin site after cleaning.

wrong with the site? The sites also were checked for the presence of mosquitoes. If the site was active the VCT III contacted a corresponding technician to treat the site. The images and information gathered were then turned into reports using Microsoft Word and Power Point. The report identified the number and location of sites reviewed and any pertinent inquiries. This information described problematic sites and allowed everyone involved to understand the site and what was observed by the VCT III. Reports were sent to the direct supervisor or manager in the department for review. After the reports were reviewed and finalized a meeting was convened in person or using Zoom with the Public Works Department for the city being reviewed. Public meetings allowed a representative from each agency to go through the sites in the field. This approach allowed the representative from the city to see what our concerns were in real time. Meetings over Zoom or other digital platforms were also successful because we used Power Point to present our data on the problem sites from the corresponding city.

During the meeting questions were exchanged between our district and the public works agency about problematic sites. Possible solutions were discussed and categorized as either long-term or short-term solutions. A long-term solution typically involves more resources, time, and planning. For example, a gutter with a depression that collects water would need construction to repair and level the gutter. This type of involvement may require multiple people, bids, and revenue prior to scheduling, construction, and completion. A short-term solution typically was something that could be done immediately with existing resources within the public works agency. The most effective solution we have incorporated in our BMPs was encouraging the public works agencies to clean stormwater drainage systems. For example, a catch basin or series of catch basins could be cleaned using a GapVax truck that can collect up to 1,500 gallons of water mixed with sand, dirt, trash, and leaf litter utilizing a large hose that vacuumed the inside of the drain system. The GapVax truck has provided excellent results and eliminated or temporarily mitigated stormwater sources such as catch basins from producing mosquitoes (Figure 1a, b, c). One of the most important things we addressed during the meetings is the estimated time for maintenance of problematic sources. This allowed the VCT IIIs to schedule follow-up visits to the sites after they have been cleaned. This is important for re-evaluating

Figure 2.—A) Siphon drain at an intersection in Palm Springs. B) Egg rafts and mosquito larvae inside the drain.

the field sites and ensuring that they were cleaned. Creating a final report based on what was done by the city agencies followed steps 2 and 3. Reports were shared with the Operations department and presented to the district's board of trustees. Monitoring these sites continued throughout the year to determine if/when conditions again became suitable for mosquito production leading to reassessment and the renewal of the reporting.

Results and Discussion

A total of 124 mosquito sources were managed within the 9 cities that collaborated with the District from 2020 to 2023 (Figure 1a, b, c). The cities with the most problematic sites were La Quinta and Palm Desert with 20 locations each and Palm Springs with 19 locations. A total of 76 catch basins were documented with all cities combined. The second most problematic source were dry wells and siphon drains. Common to all sources was the presence of stagnant water and a combination of dirt, trash, and leaf litter accumulated because of the consistent winds from the northwest end of the valley. Consistent irrigation run off continuously fills and stays trapped inside stormwater drainage sources. Additionally, we identified and learned some interesting facts about the types of stormwater drainage in the Coachella Valley. Siphon drains also known as, "down and unders", were meant to keep intersections from flooding, collect water from gutters and pass this water underground. This design relies on gravity and will always hold water after irrigation runoff or rainfall fills the site. These drains are important to identify as they will always be a mosquito source (Fig. 2a, b). Dry wells are a type of storm drain that collects rainfall and irrigation run off and redirects the water into the ground. The water is meant to percolate into the ground after being captured or directed in through pipes. Dry wells may become overloaded, not allowing water to percolate as designed. This is especially true in the Coachella Valley because the area was once a large lake, and the elevation is below sea level. The ground water table is near the surface and so there are areas that do not percolate well as a result. Another thing we learned from the collaboration is that sump areas in catch basins are designed to hold sediments and should be maintained regularly.

Conclusion

Our initial goal of collaborating with all major cities in the Coachella Valley was successful. We were able to

Table 1.—Urban stormwater sources contributing to mosquito problems, found at 9 cities within the Coachella Valley.

	Mosquito sources managed for each city							
City	Catch Basin	Dry-well	Siphon Drains	Yard Drains	Gutters/ Drainage Ditch	Channel (Wash)	Retention/ Detention Basin	Total per city
Cathedral City	2		11				1	14
Coachella	8	4						12
Desert Hot Springs	5	7			2			14
Indian Wells	3			4		1		8
Indio	10							10
La Quinta	12	6			1		1	20
Palm Desert	18	1					1	20
Palm Springs	11		7		1			19
Rancho Mirage	7							7
Total	76	18	18	4	4	1	3	124

coordinate with city officials, share our concerns, and gain the city's support. A total of 124 problematic sites in the Coachella Valley were maintained, reducing the number of mosquito sources. We learned about the infrastructure that is related to storm water systems in urban areas across the Coachella Valley. The cities became important stakeholders in our campaign to protect public health. Monitoring stormwater systems in Coachella Valley will continue so we may evaluate the impact of our daily work and collaborated efforts with best management practices.

Acknowledgements

The Coachella Valley Mosquito and Vector Control District would like to acknowledge and thank the cities; Public Works Departments of Indian Wells, Desert Hot Springs, Palm Springs, Palm Desert, Rancho Mirage, La Quinta, Cathedral City, Coachella, and Indio; and Ken Huntzinger at Veolia North America. We also thank our Indian Wells board member, Clive Weightman, for the initial suggestion.

Development and impact of a Mobile Interactive Mosquito Cart

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Coachella Valley Moquito and Vector Control District's (CVMVCD) Tabling and Outreach events primarily consisted of literature and 'giveaways' as the focal source of engagement, with few opportunities to bring additional equipment due to space and potential liability factors. Additional equipment brought along included robust and specialized equipment, such as an Argo unit, used as stationery multi-day displays at venues such as the County fair. As a result, a need was identified to develop an easier-to-use and easier-to-transport source of engagement.

The idea for a mobile interactive cart stemmed from an existing cart initially used as an ice cream cart during employee appreciation events. The ease of use, adaptability, and generally low cost to produce further supported our efforts to pursue the Mosquito Cart as an outreach resource.

The Cart has served as an addition to our current tabling setup. The Cart is decorated based on the event theme. For example, during the month of October, the cart features Halloween decorations. The design of the cart allows for removable and collapsible components, including removable doors, wheels, canopy, and signage, facilitating the use of decorations and additions depending on the event. Additionally, the use of lightweight materials allows for ease of transport and maneuvering in confined spaces.

The Cart has been used in five outreach community events so far, and we have observed a visible increase in engagement, particularly among younger audiences. Our most recent event was the National Night Out (NNO) in 2022, in which participated previously. During this event, we engaged with 60 attendees and distributed 180 items. For the 2023 NNO event, we engaged with 363 attendees and distributed a significantly larger number of giveaway items. We have additional events planned and are continually analyzing our engagement strategies.

The Cart has proven to be an essential tool in engagement during outreach events. The ability to adapt and customize the Cart to each event's theme has proved to be crucial in further pursuing the Cart as an option for outreach events. For future consideration, a focus on gathering demographics during events will be useful in determining the best use of the Cart.

Acknowledgements

We especially thank Tammy Gordon and Robert Gaona for their collaboration.

The effect of intermediate doses of Roundup® on the larval development and survival of *Culex quinquefasciatus*

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Abstract

The southern house mosquito, *Culex quinquefasciatus*, is a vector to many pathogens in southern California, including West Nile and St. Louis encephalitis viruses. Immature stages of this species are often exposed to Roundup®, a frequently used herbicide worldwide, due to surface runoff or overspraying and subsequent drifting. Previous studies have shown some negative impacts of sublethal doses of Roundup® during the developmental stages of *Culex quinquefasciatus*, such as physiologic changes and prolonged larval stage or the high mortality of larvae due to exposure to high doses of glyphosate. The present study investigated the effect of intermediate doses of Roundup® (10 mg/l and 100 mg/l) on *Cx. quinquefasciatus* immature stages. Experiments were conducted in Environmental Chambers at the CSU San Bernardino set at 27 ± 2°C, RH 70 ± 5%, and 12:12 hour L:D photoperiod.

Our results indicated larval period prolongation and delayed pupation upon exposure to the above concentrations of Roundup®. The results also indicated that as the dose the glyphosate increased, the adult emergence decreased. Oviposition experiments have shown a lower number of egg rafts were oviposited as Roundup® concentration increased. Another interesting observation was a significant increase in the adult wingspan when the exposure dose at the larval stage was increased from 10 mg/l to 100 mg/l.

Evaluating a stormwater treatment program effect on *Culex pipiens* populations: spatial analysis of catch basins and mosquito abundance in Placer County, California

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Abstract

Vector-borne diseases, primarily those transmitted by *Culex pipiens* mosquitoes, present a considerable global health risk. This study evaluated modifications to a catch basin treatment program implemented in Placer County, California in 2018, in controlling the *Cx. pipiens* population. Prior to 2018, catch basins were treated ad hoc; after 2018, catch basins were prioritized according to historical presence of standing water. Catchbasins with a history of standing water were treated on a twice-a-year schedule. A comparative analysis of mosquito counts in traps from 2006 to 2022 examined *Cx. pipiens* abundance before and after program changes. A log transformed linear regression model incorporating program implementation, year, month and nearby catch basin counts was statistically significant and explained 31% of the variation in *Cx. pipiens* abundance, with program implementation contributing significantly to the model. Linear regression models using untransformed data as well as trap data aggregated by site, month, and year also significantly predicted *Cx. pipiens* abundance, with program implementation as a significant contributor to the model. However, categorical analysis on abundance grouped into 5 categories and number of catch basins grouped into 5 categories, using a chi-square test of independence found that the 2018 alteration to the treatment program only had a significant effect on *Cx. pipiens* abundance for traps with more than 100 nearby catch basins. Taken together, these analyses strongly support a small, but statistically significant, impact of the 2018 catch basin program change in reducing *Cx. pipiens* abundance, with the effect being stronger for locations with more catch basins nearby.

Mosquito surveillance and control challenges associated with floating solar arrays

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Abstract

Floating solar arrays have become an attractive renewable energy option for municipalities, water districts, and property owners. Unutilized pond surface area can support floating solar panel arrays that produce energy and save costs. In Sonoma County, six floating solar arrays, ranging from a portion of an acre to four acres have been constructed, and the construction of a 14-acre floating array is planned for summer 2024. The Marin/Sonoma Mosquito and Vector Control District (District) was included as a partner in the design phases of three projects and discovered three additional floating solar arrays post-construction. The design of the solar panel support floats included a large opening in the center and exposed water surface area, with the float openings and water surfaces also positioned underneath the solar panels. This orientation resulted in challenging larval mosquito surveillance and control. The access walkways within the arrays mostly included floats that were 16 inches wide. The CalOSHA regulation pertaining to walkway width specifies a minimum of 24 inches. Walking on the floats submerged below the water's surface became slippery. Canadian geese frequented the floating arrays, and large amounts of their feces covered the panels and walkways and created slipping hazards. The edges of the solar panels were immediately adjacent to the margin of the walkways and presented a trip hazard. Three of the operational floating solar arrays have produced significant mosquito populations. Liquid larvicide applications via handheld equipment were difficult, precarious, and time-consuming. Granular larvicide applications to the larger arrays via helicopter have been successful; however, due to the panels covering the open water in the middle of the support floats, the helicopter had to make a second, low-altitude flight utilizing the rotor wash to force the product under the panels. Most arrays in Sonoma County utilize equipment and design features manufactured by Ciel and Tierre, a company which has plans for additional floating arrays in California and other states. Alternative solar panel support float and walkway designs are needed to achieve mosquito source reduction and allow for safe, efficient, and effective mosquito surveillance and control operations.

Yellowjacket population monitoring in San Mateo County

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Introduction

Species of wasps in the genus *Vespula*, called yellowjackets, can be a major nuisance in San Mateo County. Two primary ground-nesting species, *Vespula pensylvanica* and *Vespula alascensis*, are responsible for the largest proportion of service requests from the public that the San Mateo County Mosquito and Vector Control District (District) receives in the summer months. The number of yellowjacket-related requests can vary greatly among summer seasons, but it is not always clear whether this variation reflects actual differences in the abundances of yellowjacket populations from year to year or is the result of other factors, such as increased awareness of District services from public outreach efforts or conversations among residents.

Seasonal abundances of *Vespula* wasps are often attributed to weather patterns, but specifics and the role of other influences is not fully understood. R.M. Bohart remarked on an unusually high number of *Vespula pensylvanica* in California in 1941, suggesting “a mild winter and relatively early spring may have been partially responsible” (Bohart 1941), but this was based on personal observations without quantitative data. Reasonably, earlier warmer temperatures could allow for earlier establishment of colonies, leading to larger populations by the height of summer. In San Mateo County, monitoring data from 1963–1967 showed a correlation between heavy rainfall in the spring and a slower start to colony establishment, whereas dry springs were associated with early establishment and higher populations (Grant 1969). These findings did not hold true at all sites, and other factors were not considered.

To better understand the dynamics of local yellowjacket populations, the District began a yellowjacket monitoring program in 2022. The monitoring data was used in conjunction with data collected in the county in the 1960s and yellowjacket service request numbers to examine seasonal trends. Ultimately, monitoring data can contribute to understanding factors influencing yellowjacket abundances that can be used to optimize control methods and predict resources needed during an upcoming season.

Methods

Monitoring sites were chosen at four locations within San Mateo County. Two locations were in the northern half of the county, and two in the southern half, and all sites were chosen based on accessibility and known yellowjacket activity. The sites were: Kings Mountain school—an elementary school in a mountainous area of the town of Woodside, Portola Valley Town Center—a community center and park in a wooded suburban community, Coyote Point Park—a county park located near the San Francisco Bay, and the headquarters of the San Mateo County Mosquito and Vector Control District—located in an industrial area of Burlingame, California. At each site, four traps were placed 50-100 meters apart. The traps were standard commercial yellowjacket traps (Rescue Reusable Yellowjacket Trap, www.rescue.com), which are affordable and known to be effective at attracting yellowjackets based on trials conducted in Lake County by Urquhart and Scott (2021) and our own experience. We used liquid heptyl butyrate (Sigma-Aldrich, St. Louis, MO), applied to a cotton wick placed in a glass vial as the attractant within each trap.

Once a week from May through December of 2022 and 2023, traps were emptied of yellowjackets and the lure was refreshed. If yellowjackets were alive in the trap, the whole trap was placed in a cooler with dry ice for approximately one minute. The yellowjackets were then transferred to a plastic container and brought to the District laboratory to be sorted by species and counted.

Abundance from each site was calculated as the number of yelllowjackets of both species per site per week to create baseline numbers to be used for comparisons in future years and also analyzed by month for comparison between years. Seasonal maximum and minimum and the start date of yellowjackets appearing in traps were obtained from historical records from San Mateo County in the 1960s. The District yellowjacket service request numbers for each week from June 15 to the end of October in 2022 and 2023 were downloaded from the District’s internal data management system and plotted with yellowjacket abundance.

Results

Yellowjacket abundance in 2023 was significantly greater than in 2022 (unpaired two-tailed t-test $t_{58} = 2.87$, $p < 0.1$). The peak of the yellowjacket population in 2022 was in August, whereas in 2023 the peak did not occur until September 2023. Yellowjackets began appearing in traps in 2022 on May 19; in 2023 they first appeared in traps on June 15. In both years, yellowjackets continued to be trapped in small numbers through December (Figure 1).

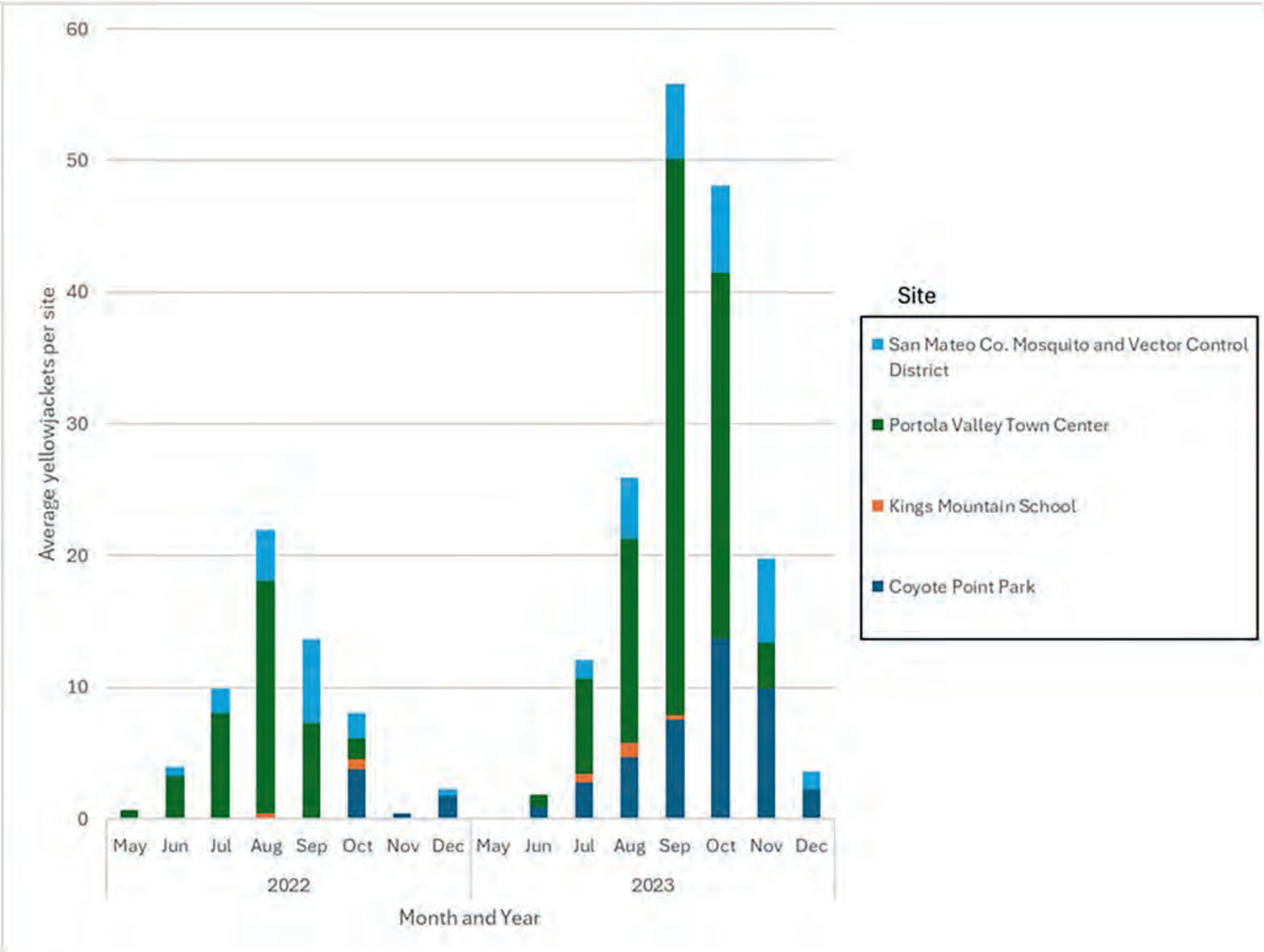

Figure 1.—Mean number of yellowjackets per site per month in 2022 and 2023. Abundance was greater in 2023.

Historical data showed that yellowjackets were trapped and recorded at monitoring sites in San Mateo County in 1966 and 1968. Similar to 2022 and 2023, monitoring data from the 1960s showed variability between years in yellowjacket abundance. However, the peak of yellowjacket abundance during the two years of the 1960s were closer temporally than during the two years of current monitoring (Table 1). Traps in the 1960s were not placed until mid-June, when yellowjackets were immediately trapped in 1966 (week of June 16) and then one week later in 1968 (week of June 22). In 1966 and 1968, traps were removed after the week of October 6, before traps counts fell to zero. Considering all four years, the start of yellowjacket activity began from May to June and decreased to a minimal level between September and November.

Table 1.—Shifts in yellowjacket populations from monitoring traps.

Trapping Milestone	1966	1968	2022	2023
First capture	No data	22-Jun	19-May	16-Jun
Highest abundance	9-Sep	21-Sep	24-Aug	28-Sep
Steep decline	21-Sep	6-Oct	9-Sep	8-Nov

The weekly number of yellowjacket service requests and the overall weekly yellowjacket abundance from mid-June through October was moderately correlated in 2023 ($r_{18} = 0.52$, $p = 0.02$). In 2022, there was no correlation (Figure 2). The total number of yellowjacket service requests in 2022 was fewer than in 2023, which matched the measured yellowjacket abundances. The week with the highest number of yellowjacket service requests was five weeks earlier than the peak of yellowjacket abundance in 2022 and six weeks earlier in 2023.

Discussion

Yearly variation in yellowjacket counts and phenology is considerable. The peak average number of yellowjackets per trap in 2023 was more than twice the peak average in 2022. Interestingly, yellowjacket foragers became active a month earlier in 2022 than in 2023, as indicated by trap counts. This does not support the idea that earlier establishment of nests allows for the growth of a higher population later in the summer. The monitoring data from 1966 and 1968 also show population variability between years, both in total

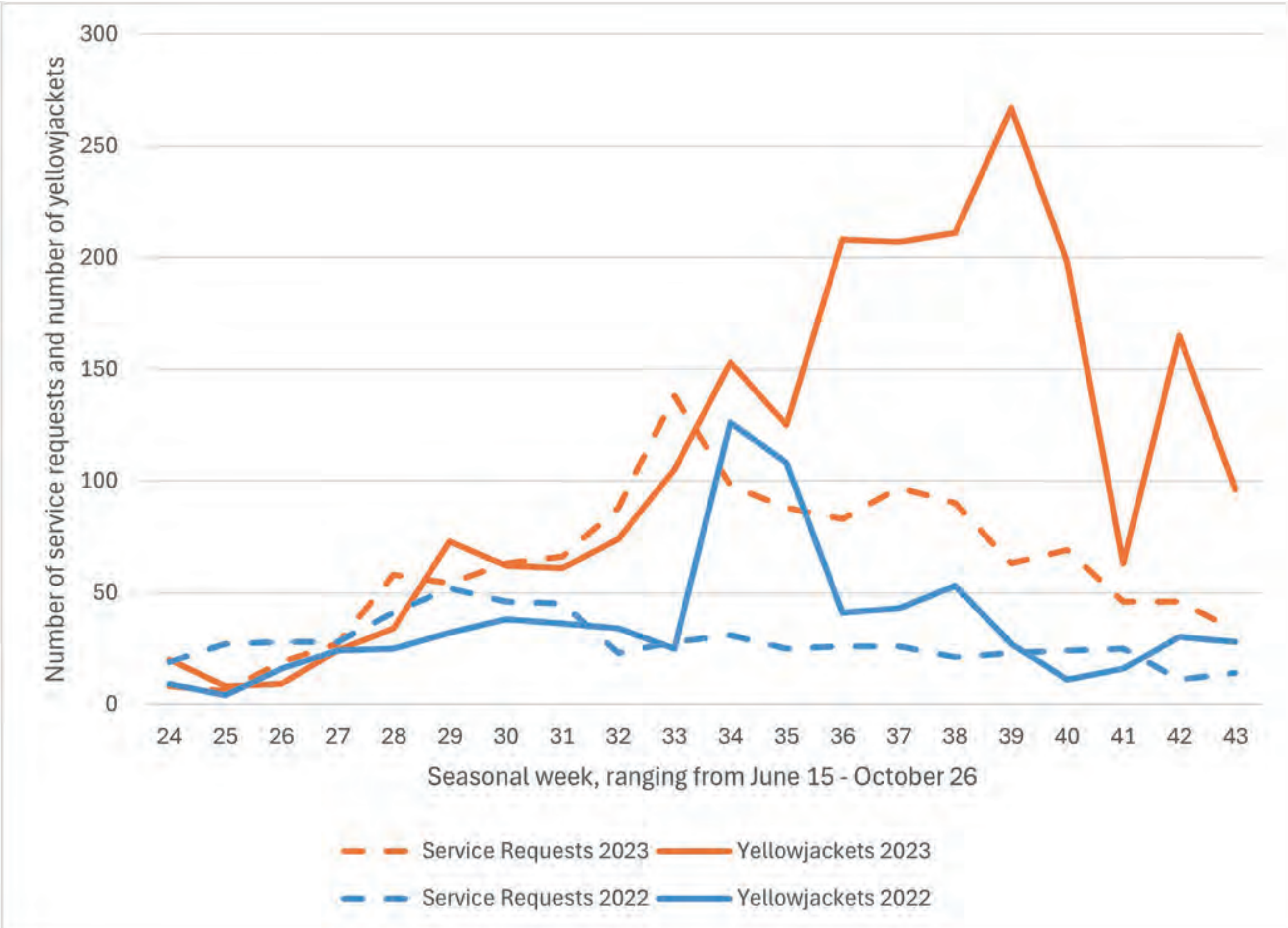

Figure 2.—Overall yellowjacket abundance and number of yellowjacket service requests. These metrics were correlated in 2023, $r_{18} = 0.52$, $p = 0.02$, but not in 2022, $r_{18} = 0.07$, $p = 0.774$.

abundance and peak population week. Unfortunately, the abundances of yellowjackets from historical data in San Mateo County cannot be directly compared because the trapping method in the 1960s was dissimilar, utilizing District-made traps with a horsemeat lure.

It is notable that yellowjacket service requests peaked earlier in the season than peak yellowjacket abundances. The reason for this is unclear. Perhaps many residents who request nest treatments have had yellowjackets previously and are familiar with the service provided by the District, and therefore call as soon as they have located a nest. Meanwhile yellowjacket populations continue to increase on non-residential land and on properties of those who do not call for treatment. This indicates that the yellowjacket nest treatments performed by the District, while of benefit to individuals, do not have much effect on the overall yellowjacket population.

Continuing to monitor yellowjacket abundances will allow for future analysis of how weather patterns, particularly spring rainfall and winter and early spring temperatures, affect the populations of both *Vespula pensylvanica* and *Vespula alascensis*. This increased understanding may help make predictions early in the season about the effort and resources that will be needed by District operations to manage yellowjackets that summer. It also would allow for early outreach about yellowjackets and related District services to neighborhoods or community locations such as schools and parks. Additionally, trials of lethal bait in high abundance sites may be evaluated in conjunction with baseline data from monitoring sites to determine the value of baiting as a control tool.

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The crucial role of strong Resident-Technician relationships in Vector Control Programs

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Abstract

Vector control technicians play a pivotal role in safeguarding public health by mitigating the spread of vector-borne diseases. However, their work is not without challenges. Some of these challenges include environmental factors, safety hazards, and community engagement. Community engagement is fostered by the relationships that the technician forges with the residents they work with. This presentation examined the significance of cultivating robust relationships between vector control technicians and residents within the communities they serve.

What's the Procedure?: Taking Surveillance Data to Social Media

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Abstract

Vector control districts across the state rely on multiple methods to disseminate important information to the residents they serve in a timely and accessible manner. Social media channels have become an essential tool, but with ever-evolving algorithms and content creators, the San Gabriel Valley Mosquito Vector Control District (SGVMVCD/District) continues to expand its reach by strategically using surveillance data, engaging partners, targeting locations, and adapting to the needs and likes of its diverse population.

West Nile virus is endemic in California, but using surveillance data to guide the District's social media efforts helps the District adapt to the changing necessities of its area. Virus positive mosquito samples can serve as opportunities to engage with member cities and stakeholders to remind them of the importance that mosquito control plays in public health. The District has utilized all available platforms to share information about mosquitoes and West Nile virus, and has engaged member cities and targeted residents using strategic ad placements. The tools developed to assist cities has amplified the District's reach and messaging.

Trials and tribulations of language translations for community outreach

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Abstract

Identifying and addressing barriers to access vector control services is necessary to equitably protect the public health of diverse communities. To identify potential barriers to accessing program services, the County of San Diego Vector Control Program analyzed program service levels across the county with respect to demographic census data and found a statistically significant association between higher levels of linguistic isolation and low numbers of service requests. To address this potential barrier, the program expanded its efforts to provide information and services in languages other than English, including Spanish, Tagalog, Arabic, Persian, Chinese, Korean, Somali and Vietnamese. This entailed translating brochures, running multilingual educational media campaigns, offering meeting translation, and ensuring that online resources were compatible with automatic translation tools such as Google Translate. Translation into so many different languages presented many challenges, including cost, space constraints, speed, accuracy and quality control, which was complicated by technical content, nuances of vector messaging and staff unfamiliarity with many of the languages. Although the Vector Control Program used County-approved contracted language translation services, it found that using departmental staff familiar with both the foreign languages and the intended meaning of the vector messages provided a second level of review that greatly increased the accuracy of the commercially produced translations. Outreach staff also simplified webpage content so that the results of Google Translate were accurate across many languages and created a list of preapproved standard phrases for official notifications to direct foreign-language readers to translated versions of the notifications posted on the web. These efforts resulted in increased engagement of foreign-language speaking members of the public. For example, media campaign digital advertisements in all non-English threshold languages averaged 59% higher click-through rates than corresponding English advertisements. Although time consuming, enhanced translation efforts provide more accessible information for diverse communities and support the goals of equitable access to vector control services.

Can you visualize it? Community science data for grades K – 8

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Abstract

Community science engages students in public health initiatives while also addressing California Next Generation Science Standards. The San Gabriel Valley Mosquito and Vector Control District's (District) community science programs encourage students in kindergarten through eighth grade to actively participate in mosquito control by clearing their properties of mosquito sources and submitting water samples from around their property to the District. Middle school students take the process a step further, utilizing oviposition cups to monitor for *Aedes* eggs. Data visualization is critical for disseminating information and creative visualization strategies must be used for such a young age group. Student samples are analyzed by Education Specialists and/or middle school students and results are posted to Padlet.com, an educational tool used by the District to display results to the community. Using the student's unique "agent number," the student can view a photo or video of their sample, whether mosquitoes were present or absent, and a description of the results. Not only does Padlet.com make views of students' samples under the microscope accessible to children as young as five and their families, it mobilizes them into taking action against mosquitoes. The samples provide an opportunity to learn to recognize mosquitoes at various life stages and encourages them to remove the sources around their homes where mosquitoes develop. Additionally, the application allows the District to export the data by tracking and mapping the presence or absence of *Aedes* mosquitoes. Community science and the data gathered provide a platform for real-life science application and supports the District's mission while increasing brand recognition and goodwill.

Introduction

Community science is an excellent avenue to engage students in public health initiatives that support the Integrated Vector Management (IVM) process and California Next Generation Science Standards (N.G.S.S.). The San Gabriel Valley Mosquito and Vector Control District (District) provides K – 12 N.G.S.S.-aligned programs to over 550 schools within the District's boundaries. Two community science programs reach distinct age ranges: Vector Inspector Program (V.I.P.) is an elementary age program whereas Operation Mosquito G.R.I.D. (Growth Reduction, Increased Detection) is a middle school program. Both community science programs encourage students to actively participate in mosquito control on their residential properties. A core component of the programs is providing students hands-on learning experiences such as the recognition of mosquitoes at all life stages, collection of stagnant water samples from their properties, and elimination of mosquito habitats. Similar hands-on mosquito education programs have been implemented in Panama City Beach, Florida (Mulla 2021) and East Tennessee (Trout Fryxell et al. 2022) by the Smithsonian Science Education Center (O'Donnell, 2021), and on a nationwide scale (Cohnstaedt, et al. 2016). However, one core element missing in previous years and in similar programs is the use of visual data to display student data from the microscope and at a community-wide level. Padlet.com (Padlet) was utilized as a public-facing online platform for student data, granting the community access to student surveillance results.

Methods

In both V.I.P. and Operation Mosquito G.R.I.D., teachers enrolled their classes for the respective program via a MailChimp form embedded on VectorEducation.org. Teachers distributed parent permission slips digitally or sent hard copies home with students. Middle school students also signed up individually using a SurveyMonkey form embedded on VectorEducation.org. This year 1,066 students were eligible to participate in both programs. Once signed up, each student was assigned an agent number to protect the privacy of all students. Then, students were provided materials, including a resealable bag, pipette, souffle cup, and educational material. Middle school students also received a larger resealable bag with an oviposition cup, oviposition paper, checklist, and calendar with deadlines for each activity. Students were tasked with inspecting their yard, collecting water samples, and clearing their yards of any potential mosquito sources. One week after water sample collection, middle school students set oviposition traps on their private properties for one week. The data was then analyzed either by District staff or by the middle school students and published publicly on Padlet. In class, water sample and oviposition paper analysis were offered exclusively to teachers trained by District education specialists.

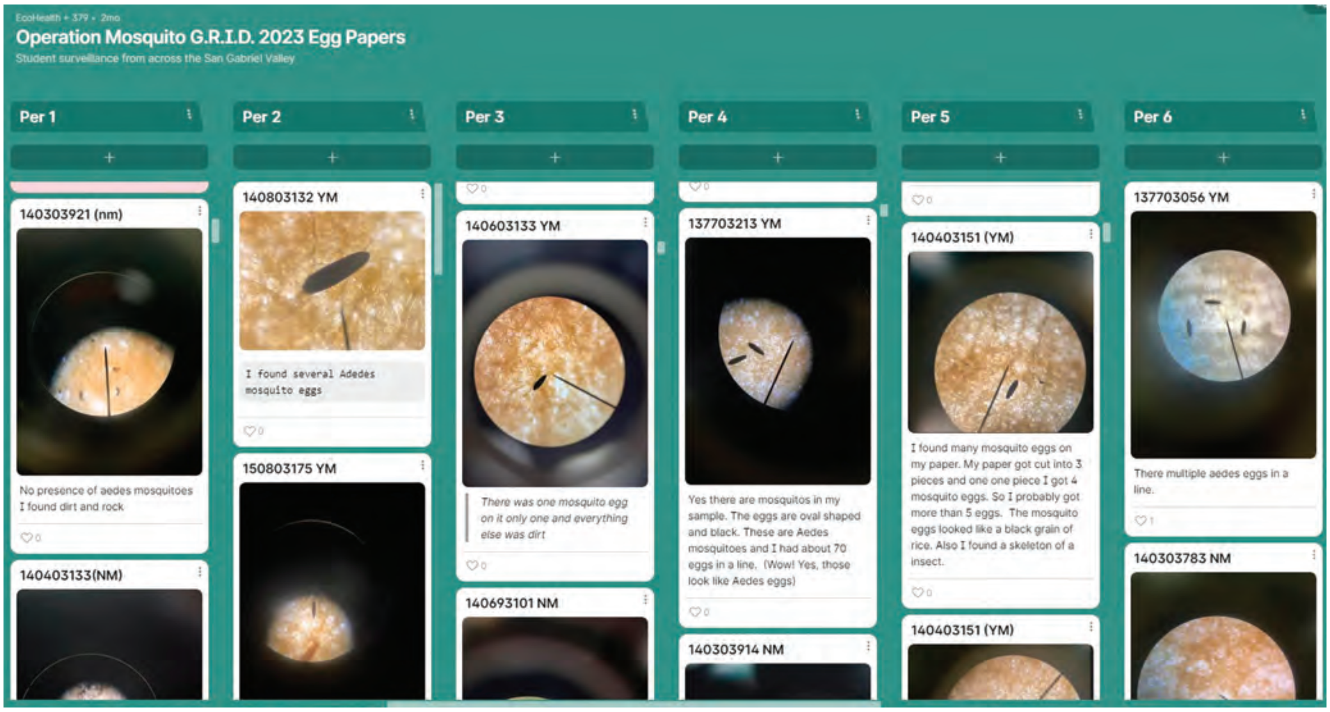

Figure 1.—Operation Mosquito G.R.I.D.: Padlet Webpage for 2023 Oviposition papers.

All student data was published publicly on Padlet. Padlet is an online bulletin board that allows users to post videos, photos, files, and links. This resource is commonly used in K – 12 education. Padlet allows for multiple useful restrictions such as delayed publishing of posts until reviewed by the owner and various options for peer commentary. The posts are hidden until District staff approves the image and description content and allows for public access.

Unique Padlets were created for each community science program and type of data: water samples and oviposition papers. Students were provided with QR Codes and links to access their respective Padlet pages. In the subject line of each post, the analyst, either a District staff member or middle school student, posted the agent number and a code (Figure 1). The code "NM" was used to symbolize no mosquitoes were found, while "YM" was used to indicate when mosquitoes were found. The body of the post included a photo or video of the sample under the microscope and a description of findings. Students were provided with laboratory handouts that guided each step of the posting process, including elements to incorporate in the description and sentence starters. Students also used mosquito keys and a key of commonly found aquatic and terrestrial micro- and macro-organisms to assist with their analysis.

Education specialists engaged with student data by providing comments written in paratheses at the bottom of students' posts to react to and guide participating students on their microscopic discoveries. Education specialists could also change the color of the post to highlight submissions that were missing critical elements and wrote in comments or instructions regarding the missing components.

The data were downloaded as an Excel file from Padlet and formatted for analysis. After combining the students' addresses from the Operation Mosquito G.R.I.D. survey data with the Padlet data, maps were created using Google My Maps. Individual maps were made for water analysis and for oviposition papers, both indicating where evidence of mosquitoes was present or absent using colored location markers. Graphs were also created to illustrate the percentage of samples containing mosquitoes.

Results and Discussion

Of the 167 V.I.P. samples returned, 12% had mosquitoes in any life stage in their water samples. As for Operation Mosquito G.R.I.D., of the 433 water samples, 18% contained mosquitoes and 27% of 364 oviposition papers had *Aedes* mosquito eggs present. The map (Figure 2) shows the distribution of the oviposition paper findings during the weeks of October 2 – 22, 2023.

Teacher surveys for 2023 also emphasized the benefits of Padlet and the opportunities that programs like V.I.P. and Operation Mosquito G.R.I.D. provide to students. In the teacher surveys, teachers stated:

> (Fifth grade teacher, 2023) "Kids loved . . . seeing the "gross" things up close."
>
> (Eight grade teacher, 2023) "SOOO many more AEDES eggs this year! It was awesome AND awful!"
>
> (Eight grade teacher, 2023) "This data is fascinating."
>
> (Eight grade teacher, 2023) "Not only did students get a good look at possible science careers, they also felt a sense of community as they worked to eliminate the threat of the *Aedes* mosquito in their community."

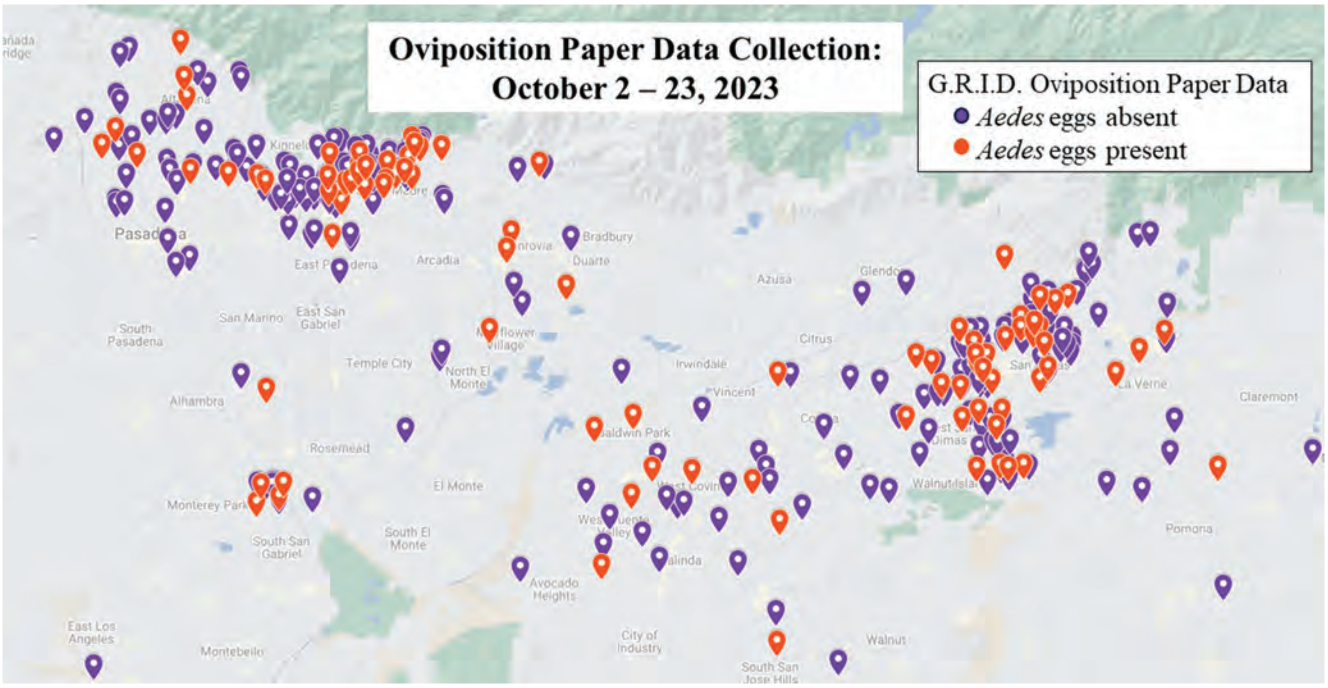

Figure 2.—Operation Mosquito G.R.I.D.: Oviposition trap results.

Based on teacher feedback, these hands-on learning opportunities successfully engaged students regarding mosquito prevention and surveillance, mosquito-borne diseases, and future career opportunities.

Programs such as MEGA:BITESS (Trout Fryxell et al., 2022) have successfully utilized ArcGIS to display student data to the public. However, the use of ArcGIS requires addresses, which we deliberately kept hidden from the public. Our choice to utilize Padlet was twofold: it allowed us to collect data without face-to-face interaction with students and provided a platform to display data to our entire community in an accessible and age-appropriate way. Padlet posts successfully displayed mosquitoes at all life stages and created a community-wide learning tool. For younger students unable to manipulate microscopes on their own, seeing these photos and videos provides opportunities to dive deeper into science that is typically out of reach at their age level.

Documenting data in an age-appropriate manner is critical to help students see their community and science in new and exciting ways. These programs make science and mosquito control interesting and reliable by tying science to students' immediate environments and provide new perspectives on the local ecosystem. Students as young as five and their families are also mobilized to actively prevent mosquitoes. Learning to recognize mosquitoes and potential sources around their own home builds self-efficacy, knowledge, and skills that are immediately applicable and useful in day-to-day life.

The use of Padlet to capture data during student class time did come with challenges. First, classrooms have limited access to time, microscopes, microscope phone adaptors, and internet for middle school students. During class, students had difficulty taking clear photos without phone adaptors. Phone adaptors were also difficult for students to use because of their novelty and due to phones with multiple camera lenses that change the focus of the photo. This led to blurry photos on Padlet. Additionally, some students did not have access to their own smart phone due to school policies or not owning a phone and used a peer or teacher's phone to capture photos. The logistics of using another person's phone during class may have contributed to less posts onto Padlet. Some students also could not access a strong enough internet connection to post their photos during class time, again, also potentially contributing to less Padlet posts. Educators should clearly discuss expectations and available materials and resources with classroom teachers to fill the gap and prepare as much as possible before middle school students analyze their own samples in class.

Conclusion

Community science programs and the data produced increased access to meaningful, applicable science opportunities that promoted mosquito control amongst residents. These programs also supported the District's mission, granting the District access to private properties without additional efforts by operations or surveillance staff. The data and samples from participating students can also be used by surveillance staff to grow colonies and monitor invasive species distribution across the district.

Acknowledgements

The San Gabriel Valley Mosquito and Vector Control District would like to thank all the teachers and students

participating in our 2023 community science programs. Their dedication makes our education programs successful and helps to protect the community from vector-borne disease.

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The Forgotten Audience—How improving internal communication can inspire fellow vector control employees

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Introduction

When it comes to communication priorities at the mosquito and vector control districts across California, Public Relations (PR) professionals report they work to ensure that the residents in their diverse communities understand specific information, including what a vector is, what residents can do to reduce the presence of those vectors on their properties, and what public health services each mosquito abatement district or vector control district provides to the residents in their communities. As PR professionals technically work for the people in their specific jurisdictions, providing residents with accurate information is an important communication priority; however, a communication priority that is just as important as educating the public is communicating to and with a district/agency/or program's own employees. Developing and implementing an internal communication plan is key to improving communication with district employees by improving the accurate information each employee receives and in turn, the employees can be inspired by the public health work they do once in receipt of that accurate information.

Methods

An internal communication plan has four key attributes: 1) support the organization's priorities; 2) drive action; 3) focus communication channels to the audience; and 4) develop effective and efficient operations plans. Research is conducted to identify the organizations' priorities, and the communication channels that are available to employees. These channels can include an emailed employee newsletter, messaging posted on a district's intranet site, messages on a bulletin board, and distribution using a messaging service such as Google Chat. The research informs survey questions that are designed to gain insight into the employees' preferences regarding communication channels and habits. Survey results are then shared during small group meetings that follow the survey and can ultimately drive action by encouraging employees to seek accurate information from supervisors or managers who are the source of the most accurate district information. The information gathered from the survey results and small group meetings is used to finetune the plan by establishing how the internal communications will be conducted and how often the messaging will be disseminated.

Results and Discussion

On November 30, 2023, the Public Affairs Department of Contra Costa Mosquito and Vector Control District (District) conducted the initial survey of the District's newly created internal communication plan. The results for multiple survey questions were as follows:

> The 32 employees who responded to the survey were most likely to read timely district information from an emailed employee newsletter (66%) and in a Google Chat (62%).
> These employees were most likely to ask a direct supervisor to clarify information either read or heard (71%) or ask a colleague (50%).
> These employees were most likely to ask a direct supervisor to answer a question or discuss a problem while on the job (78%) or a colleague (50%).

These responses indicated that the preferred communication channels to send important district information were an emailed employee newsletter and through Google Chat. Other answers to the survey provided opportunities for action. The majority of employees indicated that they preferred to receive clarification of information or an answer to a question from or discuss an on the job problem with a direct supervisor (71% clarify/78% question or problem). When the survey asked how likely employees were to ask for clarification of information or discuss an on the job problem with a colleague, 50% of the respondents chose the colleague. That revealed an opportunity to promote action in the form of changing employee behavior through small group meetings that followed the survey. During those meetings, the discussion included examples of the benefit of turning to a direct supervisor as they can provide more resources than a colleague.

Following the initial survey and small group discussions, and after the internal communication plan has been enacted for 12 months, another survey will be provided to the employees to assess the progress, any changes that are needed and any success that has been achieved to drive change and increase the dissemination of accurate district information that employees retain.

Conclusions

Vector control districts and mosquito abatement districts have long held “all-hands” meetings at which they disseminate information to employees. PR professionals are aware that this communication model has varying levels of success in employee retention of information. Implementing an internal communication plan has the potential to improve the amount of accurate District information each employee retains which can lead to additional advantages. An effective plan can provide opportunities to reduce the amount of misinformation and gossip exchanged throughout an agency by encouraging employees to seek accurate information from supervisors rather than colleagues. A plan can also allow District employees to feel heard and valued as their participation in the survey and feedback continues to inform the process. Ultimately, it is possible that a good internal communication plan can inspire fellow vector control employees and improve morale which is often among the organization’s priorities.

Surpassing the static: Video content increases engagement

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Introduction

The marketing and outreach landscape has changed dramatically in the last decade with the introduction of social media, influencers, and digital advertising. More outreach tools with measurable results have entered the marketplace which is a marketers dream, but deciding on a marketing mix to enhance engagement that has the most return on investment is much more complicated when weighing between where to place efforts and advertising dollars and anticipated impressions.

Although forms of traditional media, such as outdoor advertising using billboards and pole banners, radio, and TV may still garner high impressions and reach, digital advertising, specifically video on social media, has historically and statistically illustrated a high return on investment and an increase in engagement.

Methods

Video takes a static image to the next level by engulfing viewers in an experience. Attention-grabbing video content increases the attention of a viewer for much longer than a static post. Video content has a longer shelf-life because it is more 'reshared' and 'liked' after its postdate compared to a static photo. Video is also favored by algorithms on social media platforms, so it's viewed more than static content.

Results and Discussion

According to an online survey in June 2023 by SproutSocial.com, a social media management and intelligence tool, 66% of 1,817 consumers reported that a short-form video was the most engaging type of social media content. Similarly, a 2022 survey by Wyzowl of 528 unique respondents consisting of both marketing professionals and online consumers found that 95% reported that video marketing helped them increase brand awareness, 96% reported that they have watched an explainer video to learn about a service or product, and 51% indicated that they were more likely to share a video with their friends than any other type of content.

Based on these results, marketers concluded that there is a high value associated with using video in social media. So, how do we develop more impactful video to increase engagement on mosquito and vector control topics to increase brand awareness, encourage behavior change, and engage our audiences? Video that is short, has strong audio, clear talking points, good lighting and other angles of video not just a talking head (called B-roll) can better reach target audiences, encourage behavioral change and increase mosquito and vector control awareness levels.

Conclusions

Video has changed the marketing landscape and mosquito and vector control districts can increase engagement on social media sites by using video in their outreach efforts. Not all video is impactful. Short video that has clear audio and visuals will best increase user interest and engagement.

Acknowledgements

Thank you to the Placer Mosquito and Vector Control District for their support in effective public outreach strategies. Thank you to Mike Watson of Video Approach for leading a hands-on training with MVCAC attendees to encourage video adoption into outreach efforts for mosquito and vector control districts.

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Field trial of Natular™ G30 against *Aedes dorsalis* larvae in a tidal salt marsh habitat in Contra Costa County

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Introduction

Natular™ G30 is a granular larvicide formulation containing 2.5% spinosad as the active ingredient. Spinosad is a fermentation product derived from a naturally occurring soil bacterium, *Saccharopolyspora spinosa*, which has broad-spectrum activity against aquatic insects including mosquito larvae. It is classified by the Environmental Protection Agency as a reduced-risk material and is OMRI-listed for use on organic crops (OMRI, 2015). The Contra Costa Mosquito and Vector Control District has been using this and other spinosad-based products in limited amounts since 2009 in habitats such as flooded pastures and catch basins in rotation with other larvicide products. Our previous observations (unpublished data) indicated that Natular™ products were most effective in shallow water and tended not to spread once applied. The objective of the current study was to assess the efficacy of Natular™ G30 against *Aedes dorsalis* in a tidal salt marsh habitat.

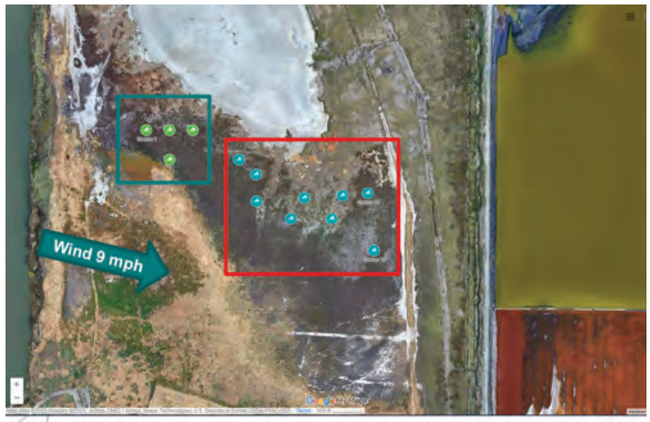

Figure 1.—Study site near Shore Acres. Green box=untreated control, red box=treated area.

Figure 2.—Granule collector.

Materials and Methods

The study site was a tidal salt marsh in the San Joaquin Delta, adjacent to the unincorporated community of Shore Acres, California (Contra Costa County). Dominant vegetation was pickleweed (*Salicornia pacifica*) and saltgrass (*Distichlis spicata*). This site produced large numbers of *Aedes dorsalis* during the previous year. Approximately 3 acres of the 10 acre site on the upwind side were left untreated as a control, and four acres were treated with Natular™ G30 by an aerial unmanned vehicle provided and operated by Leading Edge, Inc. (leaaerialtech.com) at a planned application rate of 10 lbs per acre (Fig. 1). Leading Edge also provided five collapsible granule collectors at treated sites 1, 3, 5, 7 and 9 (Fig. 2) to assess the density of granule deposition in the treated area. At the time of application, on 08 June 2023, the wind was from the west-northwest gusting to 9 mph. Larvae were collected by dipping with a standard 1-pint dipper at (or as close as possible to) 13 fixed sampling sites marked with flagged stakes, four in the control area and nine in the treated area. After finding the first larva or pupa, ten dips were collected per site. Larvae and pupae were concentrated and returned to the laboratory for identification and counting. Dip samples were taken just prior to the application, and on day 1, 4 and 23 post-treatment. As water levels varied from zero to ten inches on the sampling days, it was not always possible to collect dip samples at all of the marked sites, in which case we sampled as close as possible to the marked sites where water depth was sufficient for dipping. Pre and post-treatment counts were compared using Mulla's formula (Mulla et al. 1971) to calculate percent control.

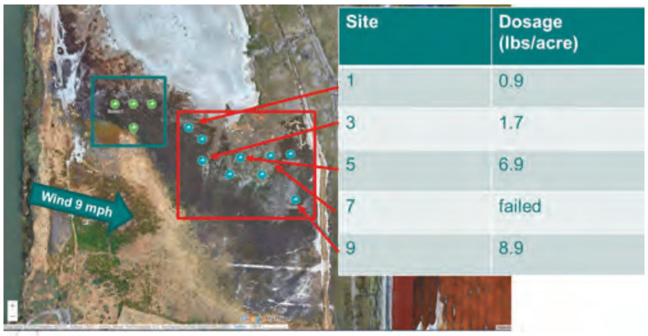

Figure 3.—Results of granule collections (measured deposition in lbs/acre).

Results and Discussion

Due to wind gusts to 9 mph at the time of application, the actual rates of granule deposition, as determined by our granule collectors, failed to achieve the target rate of 10 lbs/acre in any part of the treated area. Actual application rates varied from 0.9 lbs/acre on the west side of our treated plot (site 1), to a maximum of 8.9 lbs/acre at our easternmost sampling station (site 9) (Fig. 3). One collector at site 7 failed. Larval abundance per dip at the site were lower than we had anticipated prior to treatment, with pre-treatment counts averaging from 0.25 to 0.22 *Ae. dorsalis* larvae per dip in the control and treated areas, respectively. In spite of the suboptimal application rate, which failed to reach the targeted rate of 10 lbs/acre in any part of the treated area, we detected a substantial reduction in average dip counts in the treated versus the control area (Fig. 4). Percent control on day 1 post-treatment (9 June), as calculated using Mulla's formula, varied with the actual measured application rate, from 43% at site 1, which received approximately 1/10th of the target rate, to 100% control at sites 5 and 9, which received 70–90% of the intended dose (Fig. 5). No difference between control and treated sites was apparent after the next tidal cycle on day 23 (Fig. 4).

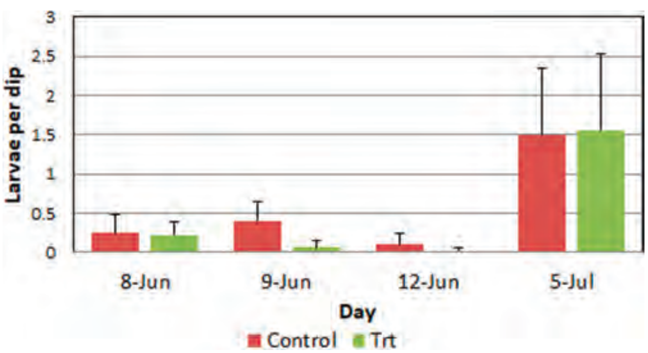

Figure 4.—Mean dip counts at control vs. treated sites before (June 8th) and after (June 9th, 12th, July 5th) treatment, with standard errors.

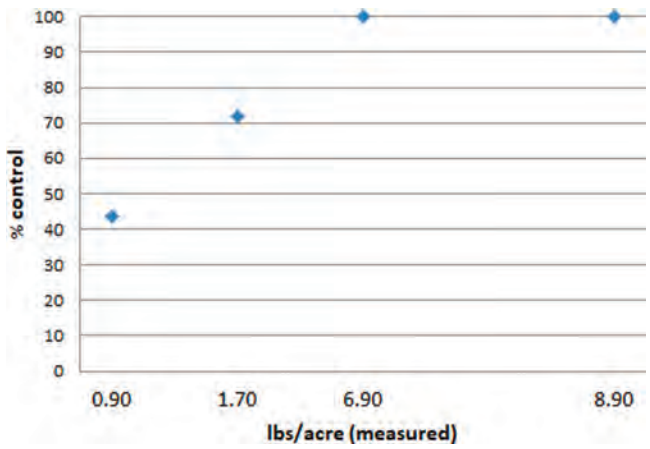

Figure 5.—Percent control (Mulla's formula), 24 hours post-treatment vs. actual application rate in lbs/acre.

Conclusions

We found that Natular™ G30 was effective for control of floodwater *Aedes* mosquitoes in a salt marsh habitat. In this field trial, the product did not provide control for a full 30 days, but that was not surprising given the tidal nature of the study site and the sub-optimal application rate. Since the product label advises that resistance to the active ingredient may develop with repeated use, we will continue to use this product in rotation with products containing other active ingredients for control of salt marsh and other floodwater mosquitoes, particularly in shallow-water habitats.

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Water quality and environmental DNA: Investigating larval ecology of *Aedes dorsalis* in the San Francisco Bay Area

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Introduction

The tidal marshes of the San Francisco Bay Area serve as important larval habitats for *Aedes dorsalis*, an important pest species and secondary vector of Western equine encephalitis virus. Management of nuisance mosquitoes in wetland habitats is complicated by their cultural and environmental importance to coastal communities (Castillo et al. 2021, Wolfe et al. 2021) therefore it is important to understand the ecological niche of *Ae. dorsalis.* Herein, we used water quality and metagenomic analysis of environmental DNA (eDNA) samples to characterize *Ae. dorsalis* larval habitats.

Materials and Methods

Water samples were collected from three locations within the tidal marsh and flats at Don Edwards San Francisco Bay National Wildlife Refuge, following inundation by high tide. Dipping surveys of the sample sites indicated that two locations contained larvae identified as first and second instar *Ae. dorsalis* (present) and none were observed at the third (absent). Laboratory analyses of selected chemical constituents of water samples were performed at the UC Davis Analytical Laboratory (detailed methods are available at https://anlab.ucdavis.edu/methods-of-analysis). Concurrently, metagenomic DNA samples were collected with 0.22 µm polyether sulfone membrane filter cartridges and DNA extractions were performed using the DNeasy Blood & Tissue Kit (QIAGEN). Novogene Co. Ltd. provided Shallow Shotgun Metagenomic Sequencing (SSMS) of samples on Illumina platforms, and taxonomic annotation based on the microNR database and MEtaGenome ANalyzer (MEGAN) software.

Results

Water Quality Analysis: Water samples from sites with larvae exhibited higher salinity and sulfate levels, but lower nitrogen content than those without larvae. Other designated parameters were not found to differ substantially (Table 1).

Microbial community eDNA: Figure 1 summarizes differences in the diversity and relative abundance of the microbial genera identified between locations where *Ae. dorsalis* were detected ("Present") or not detected ("Absent"). The top 10 genera are listed while the remaining taxa are designated as "Others". Organisms belonging to *Marinobacterium* and *Mesoflavibacter* are notably more abundant in the larval water sample; based on the number of sequences attributed to each genus in the samples, they occurred at a ratio of 9:1.

Discussion

The preliminary observational data provided only a 'snapshot' of the different conditions at the marsh sites, but the difference in salinity and sulfate levels indicated that osmoregulatory ability is an important trait of *Ae. dorsalis* that enable them to survive in brackish water. Salinity is a common environmental constraint for mosquito larvae (Rejmánková et al. 2013, Kengne et al. 2019). Further experimental studies are necessary to quantify the range of salinity tolerance in *Ae. dorsalis* and its relation to factors such as temperature and nutrient availability. Additionally, the presence of bacteria such as *Marinobacterium* and *Mesoflavibacter* indicated a possible ecological association between *Ae. dorsalis* larvae and these bacterial groups which are abundant in marine environments. Comparing the microbial content of the water samples to the midgut of larvae could potentially indicate whether members of these genera are an important source of nutrition or otherwise influence the development of larvae (Dickson et al. 2017, Rivera-Pérez et.al 2018). Although these characteristics of aquatic habitats are expected to impact larval populations, these do not account for other external influences on mosquito presence and abundance such as oviposition behavior or seasonal shifts.

Conclusions

The effects of water quality and microbial associations on larval mosquito populations are widely studied but requirements vary dramatically across mosquito genera and species (Avramov et al. 2023). By assessing the relationship between salinity, nutrient availability, and interactions with aquatic microorganisms and *Ae. dorsalis* larval development

Table 1.—UC Davis AnLab Water Quality analysis report done on 15 Sep 2023 providing a detailed summary of water quality parameters observed in larval habitats of *Aedes dorsalis* within tidal marshes in Fremont, California collected on 31 Aug 2023. The table includes measurements of salinity, reported as Estimated Soluble Salts by Electrical Conductivity (EC) and Sodium Adsorption Ratio (SAR), and concentrations of sulfate, ammonium, and nitrate.

Sample #	Larvae observed (Y/N)	pH	EC (dS/m)	SAR	Ca (Soluble) (meq/L)	Mg (Soluble) (meq/L)	Na (Soluble) (meq/L)	Cl (meq/L)	B (Soluble) (mg/L)	HCO3 (meq/L)	CO3 (meq/L)	NH4-N (mg/L)	SO4-S (Sol S) (mg/L)	NO3-N (mg/L)
1	Y	7.41	46.8	56.5	17.7	93.3	421	472	3.30	5.1	< 0.1	0.07	814	< 0.05
1 - duplicate	Y	7.66	46.6	56.4	17.8	93.0	420	484	3.30	ISM	ISM	0.09	812	0.06
2	Y	7.63	50.9	58.1	21.2	103.4	459	518	3.29	5.9	< 0.1	0.08	880	< 0.05
3	N	7.73	39.4	50.8	14.8	77.6	345	378	2.77	4.4	< 0.1	0.14	678	0.45
3 - duplicate	N	7.72	38.8	50.4	14.6	77.2	341	390	2.76	ISM	ISM	0.12	674	0.45
Method Detection Limit:		0.01	0.01	0.1	0.01	0.01	0.01	0.10	0.01	0.1	0.1	0.05	0.1	0.05
Blank Concentration:		–	–	–	0.00	0.00	0.00	0.00	0.00	0.0	0.0	0.00	0.0	0.00
Standard Ref as Tested:		6.45	0.27	1.3	0.78	0.65	1.13	0.35	0.09	1.50	–	18.85	11.3	22.24
Standard Ref Acceptable:		6.45±0.06	0.26±0.03	1.3±0.1	0.79±0.04	0.67±0.05	1.14±0.12	0.34±0.03	0.08±0.04	1.47±0.2	–	17.8±2.55	11.7±0.4	21.2±2.40

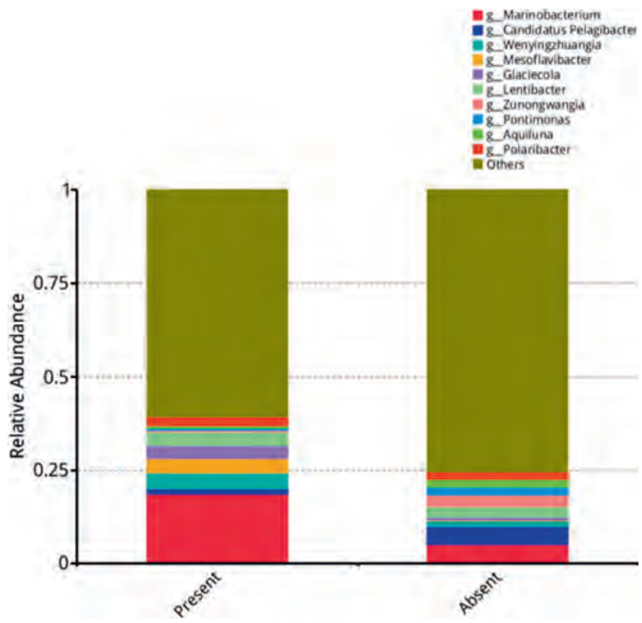

Figure 1.—Comparative analysis of bacterial diversity in aquatic environments with and without *Aedes dorsalis* larvae. Illustrates the top ten genera identified by Shotgun Metagenomic Sequencing (SSMS) of eDNA extracted from water samples.

future research can aid in the prediction of species response to environmental changes.

Acknowledgements

Thank you to everyone at Alameda County Mosquito Abatement District for their guidance and support. ChatGPT-4 (Open AI 2023) was used to develop an outline of the Extended Abstract using images from the PowerPoint slides presented at the 92nd Annual MVCAC Conference and to identify prior publications that could be referenced herein.

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An overview of the aerial adulticide and aerial larvicide program of The Upper Butte Sink Wildlife Area of the Butte County Mosquito and Vector Control District

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Abstract

Butte County has a mix of land uses ranging from rice, orchards, pastures, agricultural, and managed/natural wetland wildlife conservation. Large swaths of the county are flooded throughout the year to facilitate these activities and, in doing so, create mosquito larval habitat which is a challenge to manage effectively from the ground or along the edges of large, flooded tracts of land.

To mitigate public health threats from 50,000 acres of managed wetlands and an average of 100,000 acres of rice effectively, Butte County Mosquito and Vector Control (BCMVCD) utilizes 3 aircraft with regular routines for spraying larvicides and adulticides over the District's service area. These functions are facilitated through a standing abatement order on state lands and MOUs currently held with state and private owned wetland managers/owners. Areas are scouted and mapped on the ground by technicians, and these maps are uploaded into Mapvision and SatLoc G4 used by the pilot to apply pesticides.

As the season progresses and the rice and wetlands become overgrown, larvicide applications become ineffective due to the inability of the product to reach the target water source. As a final control measure, our aerial adulticide program applies adulticide over areas with a high density of pestiferous mosquito vectors. In the event of a public health emergency, plans for an application over a densely populated town or city using a two-engine aircraft are available, but rarely used.

This presentation summarized the aircraft, pilot, loader trucks, abatement orders, MOUs, laws and regulations, mapping systems, drift modeling technology, pesticides and maintenance of equipment used within BCMVCD's aerial larvicide and adulticide program of the Upper Butte Basin Wildlife area.

Black fly management in Greater Los Angeles County Vector Control District: A game of leapfrog

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Introduction

Black flies (Diptera: Simuliidae) are small, robust flies that are easily identified by their humped back thorax and are otherwise known as “Buffalo gnats”. They can have 7–11 larval instars before pupating. As larvae, they are important detritivores and filter feeders, which are often found attached to various substrates, such as trailing vegetation, rocks, leaves, and other debris in fast, flowing water.

Within the Greater Los Angeles Country Vector Control District (GLACVCD), the Los Angeles (LA) River has multiple inflows from natural tributaries, such as the Arroyo Seco Creek and the Big Tujunga Wash. In addition, inflows from urban runoff and local water reclamation plants have together contributed to the rich riparian corridor within this urban landscape. Multiple black fly species, such as *Simulium tescorum* Stone and Boreham, *S. vitattum* Zetterstedt, *S. argus* Williston, and *S. piperi* Dyar and Shannon, have been collected by the District in response to complaints of an infestation of “biting” flies (Pelsue, 1970). Though *S. vittatum* does not readily feed on humans, it will swarm in large numbers, and therefore is a nuisance species of concern in the LA River. Also of concern is the anthropophilic species *S. tescorum*, which is a regular inhabitant in tributaries that flow through the hills and canyons in the northeast portion of the District’s boundary.

As a result of a severe black fly infestation along the LA River corridor that impacted residents, businesses, equestrian activities, and sporting events, the District created its first Black Fly Control Program in 1996 through District Resolution 96-7. The resolution established a special assessment zone along an 18-mile stretch of the LA River for properties within a 2-mile buffer of the river and its tributaries. Within this special assessment zone, property owners are charged a slightly higher assessment fee to cover the costs of surveillance and treatment. Currently, the annual assessment charges an additional $ 0.33 per parcel. Additionally, the District will treat other impacted areas outside the special assessment zone on an as needed basis.

Methods

Beginning in March, personnel from the GLACVCD’s Science-Technical Services department (Sci-Tech) commenced weekly surveillance activities, which continued through the month of November. Sci-Tech staff surveyed 6 sites along the LA River and the Arroyo Seco Creek for substrates harboring immature black flies. Other stream systems were treated on an as-needed basis generated by observed elevated black fly activity or by public requests for service. Presence and ranked abundance levels were reported to the Operations department so that treatments can be initiated when necessary.

When treatments are required, the application of the biorational product VectoBac 12AS at 7-day intervals has been shown to be the most effective method for immature black fly control (O’Conner et al., 2001). The active ingredient is the bacterium *Bacillus thuringiensis israelensis* (*Bti*), which is known for its potency against filter-feeding larvae (Molly, 1990). The District has 43 treatment stations positioned at half mile intervals. Dosage rates (18–25 ppm/min) are determined by calculating the stream discharge rate, which is a measure of a volume of water moving through a specific area in a given time. The discharge rate requires measurements of the cross-sectional area and the average flow rate. The cross-sectional area is calculated using the width of the stream or river, and five measurements of depth. The average water velocity is measured using a MFP51 Stream Flowmeter (Geopaks, Okehampton, UK), or by measuring the time it takes for a float to cover a specific distance.

Results and Discussion

Since the inception of the program, black fly abundance has not approached pre-program levels of activity, as evidenced by the lack of visible adult activity in the air and public complaints. Adults are most abundant during the warm spring and mid-summer months, but their populations quickly drop off from mid to late summer. This is largely due to the District’s treatment schedule in addition to a decrease in dissolved oxygen concentrations in the LA River as water temperatures rise. Additional late season treatments during October and November occur if weather conditions are conducive to continued black fly activity.

Conclusion

Although GLACVCD's current black fly program has had success in alleviating the economic and political impact of black fly infestations along the LA River, its Sci-Tech department actively seeks alternative methods to improve its surveillance system. The current surveillance program relies on subjective observations and training is conducted in an apprentice-like manner; however, Sci-Tech is actively seeking an approach to better quantify our observations and report results. This will allow us to produce a Standard Operating Procedure for future staff and provide a more consistent and precise data count. To that end, we are exploring the use of artificial substrates that are inexpensive, easily deployed, and provide a high-contrast surface that lends itself to an expeditious count. The District will continue to maintain a close and cooperative relationship with both the Sci-Tech and Operational staff to continue to improve the program and to prepare for potential expansions of the special assessment zone.

Acknowledgements

We would like to thank the District's Sci-Tech and Operations staff for their cooperation and hard work. We especially would like to thank retired GLACVD staff members, Paul O'Connor, Wesley Collins, and Frank Ochoa, for their dedication to developing this program.

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Vector Management Plans: Achieving compliance through collaboration

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Introduction

In the fall of 2022, the Orange County Mosquito and Vector Control District (OCMVCD) implemented its reimagined Vector Reduction Program (VRP) aimed at identifying significant vector sources and developing vector management plans. The VRP emphasized the responsibility of property owners and land managers to reduce/eliminate vector breeding sources and/or harborage, conduct routine vegetation management, and maintain access for inspection and treatment of mosquitoes. OCMVCD utilized its Vector Reduction Manual (2010) as a guide to collaborate and negotiate with landowners and land managers.

Methods

The OCMVCD established a core team to collaborate on its initial project of identifying significant vector sources in large-area sites. The team consisted of two directors, one biologist, one supervisor, and one coordinator. Through documenting evidence by collecting specimens and engaging in proactive communication, OCMVCD established vector management plans that outlined specific actions for physical control of mosquitoes and fleas on private and public properties. To date, the VRP has fostered enhanced communication with many large landowners/managers including Caltrans, OC Parks, OC Public Works, the California Department of Fish and Wildlife, academic institutions, several cities, land conservancies, HOAs (homeowner associations), and mobile home parks, resulting in a significant reduction in labor hours and decreasing risks to staff and the public. The risks to staff have included injuries while performing physical control of mosquitoes. Risks to the public have included mosquito bites and the potential transmission of mosquito-borne pathogens such as West Nile virus.

Results and Discussion

The VRP successfully shifted the burden of land management (source reduction) to those who own and/or manage properties, reducing OCMVCD resources and minimizing risk (Figs. 1–2). This transformative program illustrated the power of collaboration, teamwork, and efficient processes in achieving a shared responsibility for mosquito and vector control. The VRP established a system of shared responsibility for vector control and demonstrated that a well-crafted document can be more

Figure 1.—OCMVCD identified this OC Public Works Flood Control channel as a significant mosquito source due to persistent water and unmaintained vegetation.

Figure 2.—OCMVCD and OC Public Works negotiated a county-wide vector management plan that resulted in routine vegetation management of channels such as shown above.

effective in combating mosquitoes and other vectors than traditional methods. OCMVCD met its goals and reduced vector abundance, pesticide use, staff time spent at sites, and avoided formal enforcement actions with landowners. Vector management plans were successfully implemented at 43 locations by the end of 2023.

Conclusions

OCMVCD transformed its Vector Reduction Program from 2022 to 2023. The VRP achieved its goals through teamwork, collaboration, and negotiation. In 2024, the VRP will continue to implement vector management plans for large-area mosquito sources. The program will also expand to include significant vector sources that harbor *Aedes* mosquitoes on residential properties.

Acknowledgements

We want to thank the OCMVCD Trustees and staff for supporting the Vector Reduction Program. OCMVCD thanks the landowners and land managers of Orange County for collaborating and negotiating the vector management plans.

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Evaluating rodent interactions with bait tubes used for flea control

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Abstract

Plague, a flea-borne disease caused by the bacterium *Yersinia pestis*, is maintained in California in a complex cycle involving multiple rodent hosts and fleas. Occasional epizootic activity in public use areas typically requires intervention, often using insecticides to reduce flea densities on rodent hosts. This usually involves dusting rodent burrows, but can also be supplemented with insecticide-treated bait tubes to target rodents outside of their burrows. An impediment to bait tube efficacy is that larger rodents such as California ground squirrels (*Otospermophilus beecheyi*) may outcompete smaller rodents (e.g., chipmunks) for access to the bait tubes and limit insecticide exposure for smaller rodent species. To address this problem, we evaluated whether a modified bait tube (smaller diameter with bait dispenser) can effectively exclude large rodents while maintaining or increasing use by smaller rodents. From August to September 2022, paired bait stations (i.e., current and modified styles) were placed in triplicate for 10 days at three locations in the greater Lake Tahoe area and were rebaited every 2 to 3 days. Rodent interactions with bait tubes were recorded with camera traps and behaviors relevant to flea control (e.g., entering tube, time spent in tube, tube dominance) were classified. Generally, rodents were more attracted to the modified bait tubes regardless of species, but preference (e.g., more entries and time spent in tube) was strongest in smaller rodents. California ground squirrels made fewer tube entries likely due to the reduced tube diameter. The use of camera traps confirmed other anecdotal observations associated with bait tube use. Regardless of location, bait tube use by rodents peaked from 3 to 6 days after initial placement and rodent use typically increased after rebaiting. Although further evaluation is needed, these results help to inform the selection and placement strategy of bait tubes depending on local rodent species and to increase the utility of bait tubes for rodent flea control.

Anaplasma phagocytophilum in western black-legged ticks in Alameda County, California

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Introduction

In California, the western black-legged tick (*Ixodes pacificus*) is the primary vector of several infectious diseases such as Lyme disease, hard tick tick-borne relapsing fever, and human granulocytic anaplasmosis (HGA). HGA is widely distributed in the United States and *Anaplasma phagocytophilum (A. phagocytophilum)*, the causative agent of HGA, has drawn attention because of an increase of reported human cases (CDC 2024). In California, the number of reported HGA cases is low but widespread, and multiple cases have been described in the highly populated Bay Area counties (CDPH 2024). Alameda County Vector Control has conducted a tick surveillance program for over 25 years to monitor recreational areas across the county for the presence of pathogens in ticks. In this study, we assessed the prevalence of the rickettsial pathogen *A. phagocytophilum* in *I. pacificus* ticks from trails in parks and open spaces.

Methods

Ticks were collected by dragging a 1 m^2 white cloth flag over vegetation and leaf litter along trails. Tick collections occurred monthly from January to July in 2022 and 2023. Study sites were in parks or open spaces throughout the county. Habitats varied from chaparral dominated by *Baccharis* spp. and grassland to coast live oak (*Quercus agrifolia*) and bay laurel (*Laurus nobilis*) woodland and redwood forest (*Sequoia sempervirens*). Ticks were stored in 95% ethanol and examined with a dissection microscope to identify species, sex, and life stage. Adults were combined into up to 5 ticks per pool and nymphs up to 2 ticks per pool. *A. phagocytophilum* prevalence was calculated as minimum infection prevalence (MIP) under the assumption that there was one positive tick per positive pool.

DNA was extracted following the manufacture's protocol (MagMAX™ DNA Multi-Sample Kit, ThermoFisher Scientific, MA) and stored at −30°C until molecular analysis. Quantitative polymerase chain reaction (qPCR) was performed utilizing primers and fluorescent hybridization probes developed to specifically identify *A. phagocytophilum* (Courtney et al. 2004).

Results

In 2022, 2,014 *I. pacificus* ticks (1,340 adults and 674 nymphs) from 35 sites were tested in pools for the presence of *A. phagocytophilum*. In 2023, 1,670 *I. pacificus* ticks (1,186 adults and 484 nymphs) from 25 sites were tested. At least 25 ticks were collected and evaluated from all locations, except for two sites. In total, the *A. phagocytophilum* MIP was 1.0% (24/2,526) in adult and 0.5% (6/1,158) in nymphal western black-legged ticks (Table 1).

The county-wide MIP in adults was 0.8% (2022) and 1.1% (2023) and in nymphal ticks was 0.6% (2022) and 0.4% (2023). We identified *A. phagocytophilum* infection in ticks from 13 sites, but only four locations were positive during both 2022 and 2023 (Figure 1). Three of these four sites were in Joaquin Miller Park.

In 2022-2023, *A. phagocytophilum* minimum infection prevalence in ticks from Joaquin Miller Park was 2.3% (13/569) in adult and 1.3% (5/376) in nymphal western black-legged ticks (Table 1). These results indicate that the MIP in Joaquin Miller Park was greater than twice the prevalence observed county-wide.

Discussion

This study is the first evaluation of western black-legged adult and nymphal ticks from Alameda County for *A. phagocytophilum.* Test results from more than 3,500 *I. pacificus* showed that *A. phagocytophilum* infections in ticks was uncommon and that prevalence varied across the county. We screened *I. pacificus* ticks from all study sites

Table 1.—Prevalence of *Anaplasma phagocytophilum* in adult and nymphal western black-legged ticks (*Ixodes pacificus*) collected in Alameda County in 2022 and 2023.

	Adult *I. pacificus* No. positive pools/No. ticks		Nymphal *I. pacificus* No. positive pools/No. ticks	
Locations	2022	2023	2022	2023
County-wide	11/1,340	13/1,186	4/674	2/484
Joaquin Miller Park	5/219	8/350	4/226	1/150

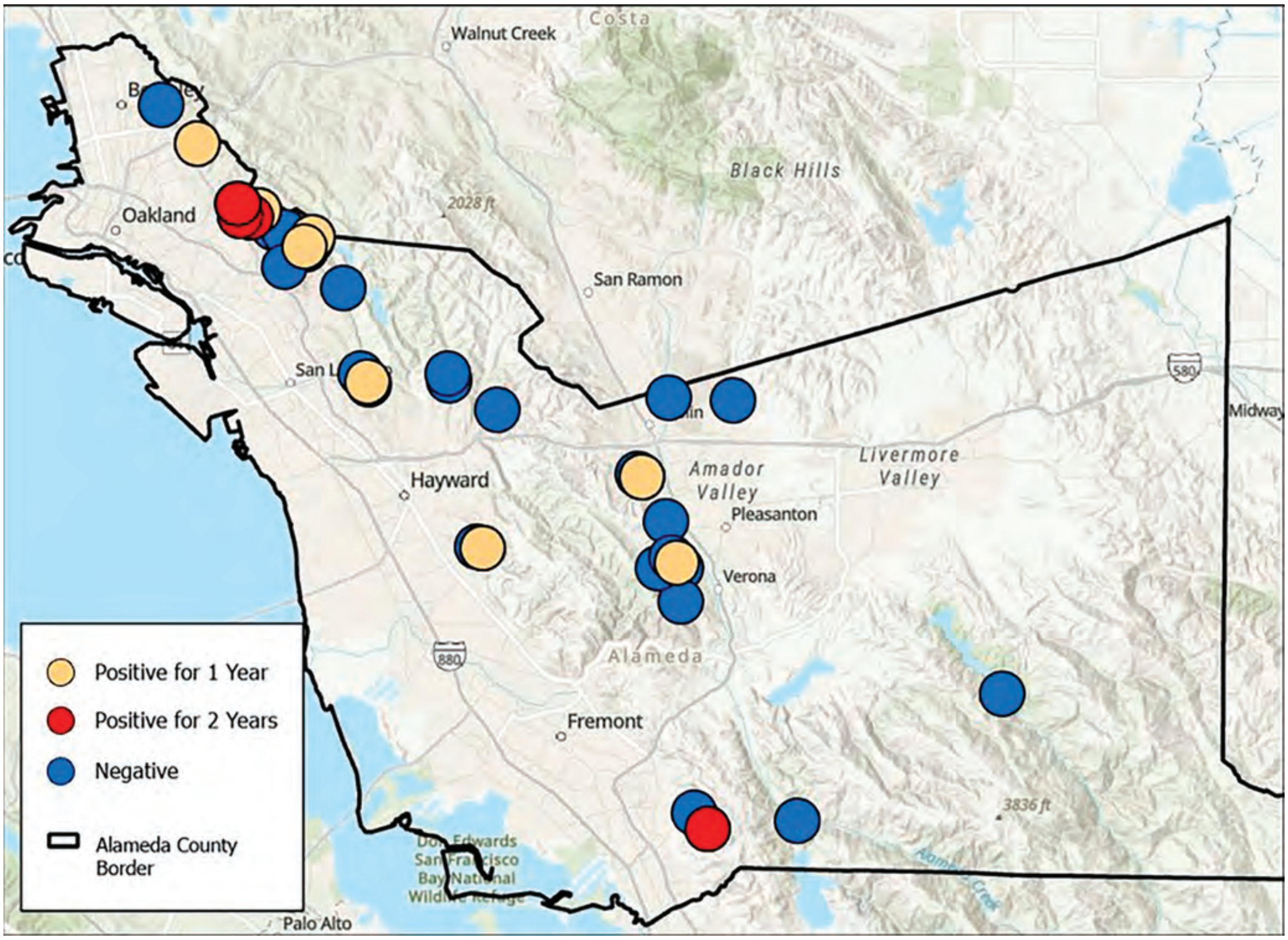

Figure 1.—Study sites and locations where western black-legged ticks tested positive for *Anaplasma phagocytophilum* in 2022 and 2023.

that were included in the county's tick surveillance program to evaluate the risk of exposure to *A. phagocytophilum*.

A study conducted in the San Francisco Bay Area in 2012 and 2013 (Nieto et al. 2016) found that *A. phagocytophilum* infection prevalence in nymphal ticks (4.2%) was higher than in adult (0.8%) western black-legged ticks. In 2021 and 2022, CDPH Vector-Borne Disease Section reported the *A. phagocytophilum* infection prevalence in ticks from Marin, Contra Costa, and San Mateo counties was in the 0 – 5.9% range (CDPH VBDS reports 2021, 2022). Those results showed that *A. phagocytophilum* was present in *I. pacificus* tick populations from the San Francisco Bay Area including Alameda County even though the distribution of the pathogen was sporadic and might vary from year to year.

Our results indicated that *A. phagocytophilum* was maintained at a specific area in Joaquin Miller Park in Oakland. Previous studies showed that far western North American communities in which *A. phagocytophilum* is maintained are complex ecologically, with a variety of reservoir hosts, multiple *Ixodes* spp. vectors, and several different strains of the bacterial pathogen (Foley et al. 2011). Further research is necessary to assess the human exposure risk to *A. phagocytophilum* on the trails in Joaquin Miller Park and in Alameda County.

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Adapting to evolving needs: Tick-focused advancements in CalSurv

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Abstract

With an enduring commitment to meeting the needs of our user community and stakeholders, CalSurv has broadened its scope to include enhanced features for tick surveillance, visualization, and data management. This presentation guided users through this new, expanded terrain, clarifying the unique aspects of tick-focused operations within the CalSurv system. It also served as a transparent update to our stakeholders, highlighting the strides we've taken in product development to adapt to emerging public health concerns.

One Health, One World: The changing epidemiology of Rocky Mountain spotted fever in California

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Abstract

Rocky Mountain spotted fever (RMSF) is a life-threatening tick-borne disease. In California, RMSF is rare; nonetheless, changes over the last 40 years in the epidemiology and eco-epidemiology of the now known vector ticks, *Dermacentor*, i.e. spp and *Rhipicephalus sanguineus*, sensu lato, have impacted our understanding of risk of exposure to this disease. These ticks occur in completely different habitats (sylvatic and peridomestic, respectively) resulting in different exposure risks for humans. In this presentation, the demographic, exposure, and clinical aspects associated with the last 40 years of reported RMSF cases to the California Department of Public Health (CDPH) were presented. Cases of RMSF in California result from both autochthonous and out-of-state exposures. During the last 20 years, more cases reported exposure in Southern California or Mexico than in the previous 20 years. The driver of these epidemiologic changes is likely the establishment and expansion of *Rhipicephalus sanguineus* sensu lato ticks in Southern California and on-going outbreaks of RMSF in northern Mexico. Recent human cases and studies have demonstrated that the role of dogs as the disease reservoir and the brown dog tick as vector need to be considered in designing prevention messaging.

Introduction to 3D printing

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Introduction

For decades, entomologists and vector control professionals have designed and built their own specialized devices for many purposes such as monitoring insects and evaluating pesticide applications. These devices are often a product of someone's ingenuity, design, and building skills. Examples of these include: aspirators made by modifying vacuums (Doğramaci et al., 2011; Thingiverse.com); mosquito breeders made from two peanut butter jars (Dees et al., 2011); and gravid traps made from tool boxes (Cummings et al., 2023). In-house or do-it-yourself manufacturing is becoming more accessible with the arrival of increasingly affordable 3D printers. This, along with the closure of BioQuip, North America's largest source of entomological supplies, in March 2022, has incentivized agencies to search for their own manufacturing solutions. The presentations from the 3D printing symposium during the 2024 MVCAC annual meeting are evidence that vector control professionals are taking advantage of 3D printing to create custom products to further their vector management programs. This is a summary of some resources available for those that want to build their own 3D printing program.

Discussion

There are many 3D printer brands and models on the market that range from $300 to $100,000+. Buyers have multiple choices on which printer to get. To start, the author recommends reaching out to current users for their input, such as the speakers from this symposium. Buyers may want to consider investing in features that will reduce time spent tinkering and recalibrating. For example, a machine with automatic bed leveling and high-quality construction may save time in the long run. Along with positive product reviews and sturdy construction, consider whether the product has affordable and available replacement parts. If there are proprietary parts that are sold only by the manufacturer, an owner may get stuck if the printer is no longer supported or if the company goes out of business. This is the same with the software. Consider whether the machine is compatible with multiple software packages to provide flexibility. There are many trustworthy open-source options that a potential buyer can use. The author expects the price for a reliable printer should start around $1000 but may be more expensive if extra features are desired.

Once a printer is chosen, there are a selection of workflow settings and filament types to consider with the intention of optimizing the final printed product. Many summaries of how to start a 3D print and what settings to choose are available on the web. Here are some places to start:

How to start 3D printing and general workflow: https://www.matterhackers.com/articles/how-to-succeed-with-your-first-3d-print (Last accessed 2/12/24).

How to start 3D printing with details on slicer features: https://markforged.com/resources/learn/3d-printing-basics/how-do-3d-printers-work/3d-printing-process (Last accessed 2/12/24).

Types of filaments: https://all3dp.com/1/3d-printer-filament-types-3d-printing-3d-filament/ (Last accessed 2/12/24).

Resources are available for those interested in developing their own makerspace (Table 1). These are often free or open for personal use. There are also many online communities to learn and share from such as YouTube channels and Reddit communities (r/3Dprinting).

For those not ready to invest in a 3D printer, there are 3D printing services offered by private companies (Table 1). The author recommends looking for nearby makerspaces such as a local library or community college. For example, Monterey Peninsula College, Monterey, CA has a makerspace and offers classes for their art and engineering students. Another example is MatterHackers, Lake Forest, CA, a local supplier of printers and filaments. They offer product support, training, consultations, and have their own makerspace.

Conclusions

3D printing is an affordable and useful tool for developing new products and ideas. With the wealth of online resources and a growing community of 3D printing experts, it's easy to begin 3D printing quickly. Many vector control professionals have developed their own products and determined that 3D printing is beneficial, as readers will see in the papers from this symposium. Ideally, the community will continue to thrive, share ideas, and develop solutions leading to greater accessibility of entomological and vector control supplies.

Acknowledgements

Thanks to Mark Nakata for organizing the 3D printing symposium for the 2024 MVCAC annual meeting in

Table 1.—3D printing resources. The list is not comprehensive or an endorsement.

Purpose	Name or Website
Repository of models	Thingiverse - https://www.thingiverse.com/
	Printables - https://www.printables.com/
Drawing software	Autodesk Tinkercad - https://www.tinkercad.com/
	Autodesk Fusion 360 - https://www.autodesk.com/
	Blender - https://www.blender.org/
Slicer software	Ultimaker Cura - https://ultimaker.com/software/ultimaker-cura/
	Prusa Slicer - https://www.prusa3d.com/en/page/prusaslicer_424/
3D printing community	https://www.reddit.com/r/3Dprinting/
3D printing community and search engine for models	Thangs - https://thangs.com/
3D printing service	Shapeways - https://www.shapeways.com/
	Matterhackers - https://www.matterhackers.com/

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Developing a 3D printing program and preliminary results

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Abstract

3D printing is an area of rapid technological growth with applications as diverse as the organizations that incorporate them. Vector and mosquito abatement district's aim to stay on the cutting edge of technological development and many have established 3D printer programs within their organizations. Districts that have incorporated a printer program are able to produce custom tools and novel mosquito traps that rival standard designs. As with any developing project or program, 3D printing requires specific resources to operate and if left unmet can lead to a program's removal. At the San Gabriel Valley Mosquito and Vector Control District, a 3D printer program was established in January of 2023 following a predesignated structure to provide specific guidance while ensuring its continued success. This structure included three organizational categories that were determined before the program could begin: resources, training and goals. Resources included the fiscal cost of the program and time usage. $1500 was allocated for the program's initiation while individual staff dedicated to the project spent no more than 10 hours per week 3D printing. Training was necessary to effectively understand and use each aspect of the program. Standard Operating Procedures or SOPs were independently developed for each aspect of the program including software training, hardware maintenance, post print production and filament testing to name a few. Goals were defined by one-, three- and five-year benchmarks that categorized success or failure of the program. These directions provided an understanding of where the program should develop and what level of user need may additionally be required. Using this methodology, the program has been successfully integrated into agency use with several developing projects underway. San Gabriel Valley MVCD is planning to continue to use the benchmark goals established in the planning phase of this program to continue to monitor and determine the success or failure of this pilot project.

Developing a Vector Surveillance Trap (VST) as a replacement to the Encephalitis Virus Surveillance (EVS) trap

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Introduction

The original Encephalitis Virus Surveillance (EVS) trap was invented 75 years ago (Roch and Fall, 1979), and the BioQuip versions of EVS traps have been used by many mosquito and vector control agencies. These traps have several issues including inconsistent performance when alkaline batteries and aftermarket brush motors were used. BioQuip has discontinued its business and has stopped providing EVS traps and replacement parts. To address all these issues, a new Vector Surveillance Trap (patent pending), termed the VST, was designed and built by our District with a 3D printer used to produce all the plastic parts.

Methods

To overcome issues with the EVS traps, highly reliable ball-bearing motor fans and rechargeable lithium batteries were used to provide the collection suction force. The new trap was designed to collect mosquitoes before they pass through the fan, thus preventing damage to the specimens (Fig. 1). Ergonomics was considered for easy trap assembly, deployment, and retrieval.

A 28-week long field side-by-side comparison was conducted in an area where many mosquito species are known to be present. The EVS and VST traps were separated by 300 feet, and their locations were exchanged weekly between Division and Division South. Each trap at each location was evaluated for 14 weeks, i.e. 14 trap nights. Both traps were baited with CO_2 from dry ice in a BioQuip EVS dry ice bucket and were equipped with incandescent lights. Data was analyzed with generalized linear mixed models using JASP software (JASP Team, 2024).

Results and Discussion

The VST trap collected the same species composition as the EVS trap with minor variation (Table 1). The VST trap collected slightly fewer mosquitoes than the EVS did in Division South, but collected slightly more mosquitoes in Division (Table 1). On average, there was a slight difference in mosquito count per trap night between the VST and EVS traps, but the difference was not statistically significant ($df = 1{,}8$, $P = 0.345$, Fig. 2). During the 28-week long field evaluation period, no mechanical and operational issues were encountered, meeting expected reliability improvement. All mosquitoes collected by the VST traps were undamaged, providing additional benefits for species identification and pathogen testing. All our staff prefer the new VST trap over the EVS traps due to its reliability and simplicity of use.

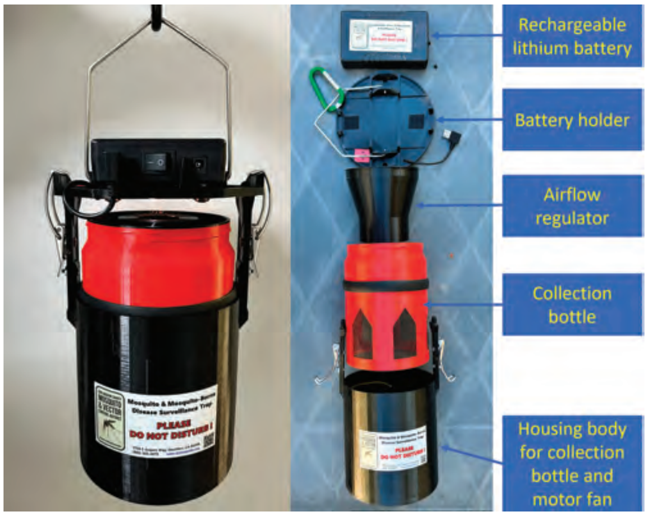

Figure 1.—The Vector Surveillance Trap.

Conclusion

Field evaluations demonstrated that the VST traps provided equal collection performance with improved reliability, consistency and ergonomics. The new VST trap is a great replacement for the traditional EVS traps, although performance in environments other than the California Central Valley requires further evaluation.

Table 1.—Comparison of species composition between the VST and EVS trap collections.

Location	Division South				Division			
Trap Type	EVS		VST		EVS		VST	
Trap Nights	14		14		14		14	
Data Type	Total	Percent	Total	Percent	Total	Percent	Total	Percent
Aedes melanimon	2,821	13.6%	5,624	23.9%	2,616	31.9%	2,306	30.4%
Aedes nigromaculis	1	0.0%	38	0.2%	24	0.3%	9	0.1%
Aedes vexans	1,161	5.6%	270	1.1%	222	2.7%	270	3.6%
Anopheles freeborni	58	0.3%	175	0.7%	20	0.2%	42	0.6%
Culex erythrothorax	36	0.2%	12	0.1%	17	0.2%	13	0.2%
Culex pipiens	544	2.6%	316	1.3%	91	1.1%	105	1.4%
Culex tarsalis	15,014	72.2%	16,058	68.2%	4,870	59.5%	4,540	59.9%
Culiseta incidens	0	0.0%	284	1.2%	7	0.1%	4	0.1%
Culiseta inornata	1,152	5.5%	760	3.2%	321	3.9%	294	3.9%
Total	20,787	100%	23,537	100%	8,188	100%	7,583	100%

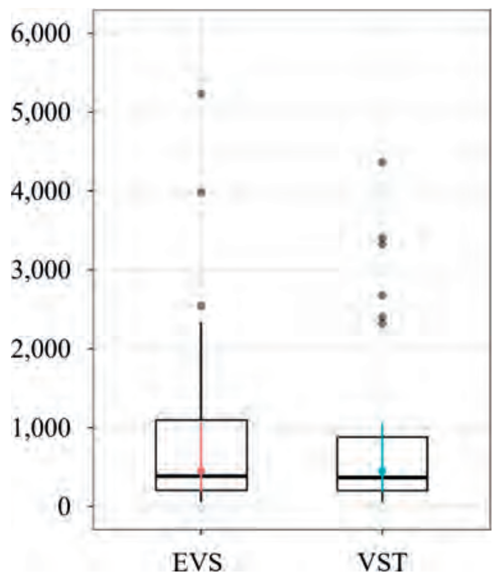

Figure 2.—Comparison of mean catch per trap night between the VST and EVS traps.

Acknowledgements

The authors thank Sumiko De La Vega and Mary Iverson of the District for helping in laboratory and field evaluation of the traps; Noah Moral of the District for the support to build the new traps.

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Using 3D printing to support programs at Placer Mosquito and Vector Control District

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Abstract

Fused deposition modeling, better known as 3D printing, can be a useful tool for making replacement, novel, unavailable or expensive equipment/parts relatively quickly and cheaply given a modest investment in equipment and expertise. At Placer Mosquito and Vector Control District (PMVCD), replacement/novel parts for drones and field operations as well as parts for laboratory and office use are routinely printed, and usually at a fraction of the cost of new parts. In addition, using computer assisted design (CAD) software, parts for various pieces of equipment such as bottle-rollers and 96-well plate shakers can be designed and built for a fraction of the purchase price of their commercially available equivalents. However, 3D printed parts are not appropriate in all cases: for example, the plastic used for printing necessarily has a relatively low melting point and limited strength, thus printed parts might not be suitable for high-temperature environments or for parts requiring high strength.

A tool to make more tools for mosquito control

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Abstract

Mosquito control programs (MCP) rely on a variety of tools to conduct the work of Integrated Mosquito Management. Many of the tools of this public health trade are items that can be easily sourced and purchased. However, there are also several 'niche' tools used by MCPs which must be custom built, modified, or are cost prohibitive. The technology of 3D printing has become cheaper and easier to use over the past decade allowing many tools to be designed and created with a 3D printer. The Salt Lake City Mosquito Abatement District (SLCMAD) first acquired and started using a 3D printer in 2017 after learning how MCPs in New Zealand were using them to enhance their programs. SLCMAD has created tools that help in different aspects of mosquito control including printing new trap bodies, vial holders for trucks and ATV's, replacement parts in granule spreaders, new granular pesticide application equipment, parts for mosquito blood feeders, funnels to dispense pesticides from totes, parts for caged ULV spray trials, and skimmers for mosquito fish tanks. The 3D printers currently used at SLCMAD include the MakerGear M2 (https://makergear.com/), Creality CR-10 (https://www.creality.com/products/creality-cr-10-smart-3d-printer), and Elegoo Neptune 3 Max (https://us.elegoo.com/products). Of these three, the Neptune 3 Max can print the largest objects, with a printing bed able to make structures approximately 16 inches long, by 16 inches wide, by 20 inches tall. SLCMAD uses Tinker CAD (https://www.tinkercad.com) to design new objects and store designs in Thingiverse where the mosquito control community is free to access and share designs.

Using 3D-printed tools to streamline mosquito control product evaluations

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Introduction

The Research, Development, and Engineering (RDE) Department of Clarke Mosquito Control Products owns and operates three 3D printers that can quickly turn a digital model into a usable object through a process known as fuse deposition modeling (FDM). These 3D printers are used for developing and deploying tools that aid in the evaluation of the efficacy of mosquito control products. The evaluation of mosquito larvicides, both in mesocosms and in natural habitats, is aided by the use of larval sentinel cages that allow for the exposure to active ingredients in the water but contain the larvae within a desired area. FDM was used to create a larval sentinel cage that is more secure, customizable and easily assembled than previously available options.

Methods

Sentinel cages that were previously deployed by Clarke were made from 4″ HDPE irrigation pipes with windows cut into the plastic that were covered with tulle to allow water exchange while keeping mosquito larvae contained (Figure 1). The HDPE pipe cages were costly and time consuming to make, as well as difficult to create with uniform openings. To replace the irrigation pipe components, a series of concentric rings were designed in Autodesk® Fusion360™ that could be assembled easily and precisely while maximizing the permeable space of the cage. Polyethylene terephthalate glycol (PETG) was the chosen filament material due to its low cost, ease of printing and durability in aquatic environments. Tulle was replaced with woven stainless steel mesh (60 mesh size, Ovsor®) to provide rigidity to the sentinel cage (Figure 2).

Results and Discussion

The 3D printed sentinel cages have been used in seven different efficacy studies of multiple larvicides in mesocosms since their inception, with the duration of the studies ranging from one to six weeks. Biologists that conducted these studies previously had used the older HDPE pipe cages as well as mesh laundry bags and found the 3D printed cages to be easier to use than both sentinel cage options. The 3D printed cages provided more internal work space than the HDPE pipe cages, which made finding and

Figure 1.—The previously deployed sentinel cage that was made from irrigation pipe and tulle.

Figure 2.—A fully assembled 3D printed sentinel cage with stainless steel mesh.

removing dead larvae easier. The rigid structure of the 3D printed cage kept the cage from collapsing in on itself like the mesh laundry bags tended to do. Three of the studies directly compared the 3D printed cages to the mesh laundry bags and there was no observable difference in the mortality rate of the treated larvae or in the survival rate of untreated larvae.

Conclusions

Using 3D printers has allowed the Clarke RDE team to create custom solutions for evaluating mosquito control products, in this case, a sentinel larval cage for evaluating larvicides in mesocosms and natural habitats. The 3D printed sentinel cage improved on prior designs because it can be assembled uniformly and without power tools, while maintaining rigidity and durability that was lacking in mesh bags.

Acknowledgements

Clarke would like to thank Anastasia Mosquito Control District (120 EOC Drive, St. Augustine, FL 32092) for allowing the use of their concrete pools for one of the seven mesocosm studies and for guidance on the usage of mesh laundry bags as larval sentinel cages.

Leveraging 3D printing for custom solutions in vector control applications

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Introduction

As tools and methods used by vector control districts become more complex and specialized, custom designs and parts have become necessary to facilitate the adoption of new techniques. Although businesses that develop and produce some of these tools exist, they are few and far between, are often cost prohibitive, and frequently lack off-the-shelf products that fit the specific applications for a vector control district. In response, many vector control districts have adopted 3D printing to solve this problem. The use of 3D printing has surged in the last decade, resulting in decreases in costs and the influx of informational resources that have allowed hobbyists as well as industry professionals to adopt this technology. For the Greater Los Angeles County Vector Control District (GLACVCD), the acquisition and use of a 3D printer has been invaluable for many projects, as it allows for quick and cost-effective prototyping of designs and bulk printing of custom parts unavailable from retailers.

Several of the projects for which GLACVCD utilized 3D printing include: a battery brace for Reiter-Cummings gravid traps and a battery enclosure for EVS/CO_2 traps designed as a part of a project to replace all the D batteries from routine traps with rechargeable lithium-ion USB battery packs; a blood-feeding device designed for maintaining an *Aedes aegypti* colony; and plastic bushings designed to provide smooth adjustments for a Fay-Morlan pupal separator.

Methods

The 3D printer used to print all the parts described hereafter is the Dremel 3D45, using an Overture© polyethylene terephthalate glycol (PETG) filament. A nozzle temperature of 250°C and a bed temperature of 70°C was used.

The battery brace used to secure the rechargeable lithium-ion USB battery pack to the Reiter-Cummings gravid trap was designed in Autodesk Tinkercad. Dimensions for the brace were established after measuring the size of the battery as well as the inner dimensions of the trap to which it would be affixed.

Development of a custom enclosure for the rechargeable lithium-ion USB battery pack to be affixed to the EVS/CO_2 trap involved examination of various designs hosted on the open-source 3D printing repository, Thingiverse. A customizable box design (https://www.thingiverse.com/thing:1264391) was selected that could be modified in the script-based 3D modeling software, OpenSCAD. Dimensions were modified within the software to accommodate the battery, one side was redesigned with a captive door that could slide up and down to facilitate easy battery insertion and removal, and a hole was added to accommodate the insertion of a USB cable to power the trap.

Designing a custom blood-feeding system for an *Ae. aegypti* colony began with building a 3D printed prototype in Tinkercad to house a flexible heating element which would heat a 3-inch watch glass filled with blood and covered with a membrane. A separate design (https://www.thingiverse.com/thing:5923956) produced by Salt Lake City Mosquito Abatement District (SLCMAD) was also printed. This design housed a thin film heating element which would heat up an aluminum block filled with blood and covered with a membrane. Modifications of this design included drilling a small hole into the aluminum block to accommodate a small temperature probe attached to a programmable temperature controller which could keep the temperature of the heater within a defined range for optimal blood feeding.

3D printed plastic bushings were designed in Tinkercad after using calipers to measure the outer diameter of the bolts used to adjust the glass plates in a Fay-Morlan pupal separator. Eight bushings were printed and placed on both sides of the bolts around the outer glass plate.

Results and Discussion

The 3D printed battery brace (Fig. 1) in the retrofitted Reiter-Cummings gravid traps proved to be extremely reliable, keeping the battery pack upright and secure inside the trap box. All Reiter-Cummings gravid traps at GLACVCD have been retrofitted with the part and have been in constant use for the last two years.

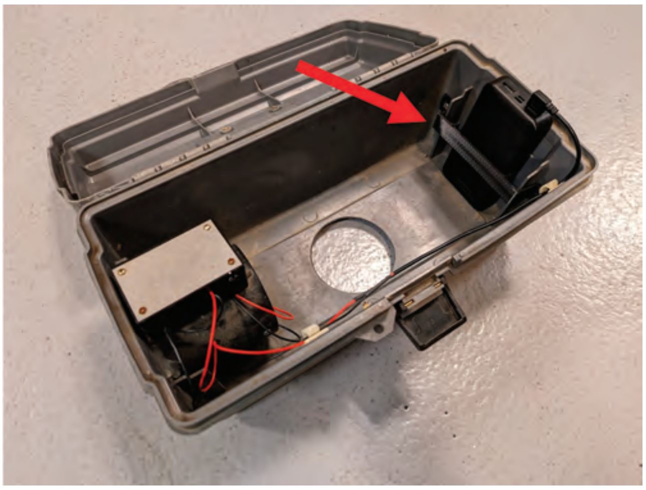

Figure 1.—Reiter-Cummings Gravid Trap with an installed 3D printed battery brace securing a USB battery pack.

The 3D printed battery enclosure (Fig 2) atop the EVS/CO_2 traps were not as successful, with multiple instances of the plastic cracking and the pieces separating from each other. This is a result of the part not being able to be printed in one piece, and relying on screws to keep the pieces together, which do not fit well into 3D printed plastic. Modifications to the design will need to be developed to ensure a more reliable enclosure for this trap.

Figure 2.—EVS/CO_2 Trap with an installed 3D printed battery enclosure containing a USB battery pack.

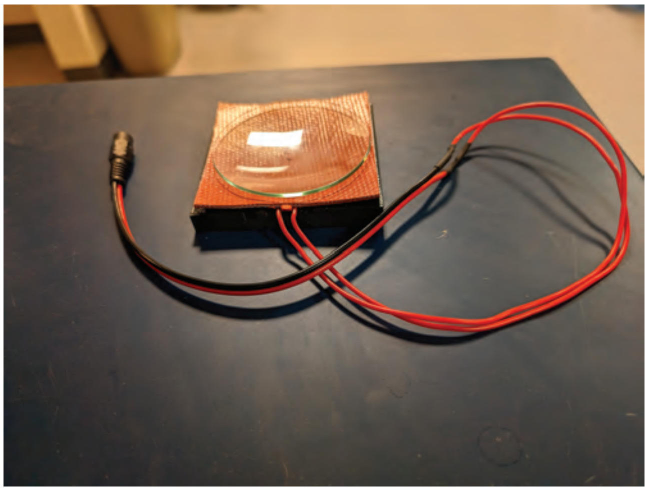

Figure 3.—Prototype mosquito blood-feeding device, with a 3D printed base, a flexible heating element, and a watch glass for blood.

The original prototype blood-feeding device (Fig 3) that was developed did not perform adequately. *Ae. aegypti* females initially seemed interested and would probe the membrane, but did not often successfully blood feed. It is thought that the depth of the watch glass may have been too shallow for blood feeding. The shallow depth coupled with the large surface area of the blood also led to the blood quickly drying out. Because this design was built around the dimensions of the watch glass, a new approach was explored. The modified SLCMAD blood feeder design (Fig 4) exhibited greater success. The blood reservoir was designed to be slightly deeper, with no blood drying problems and *Ae. aegypti* females fully engorged on this presentation.

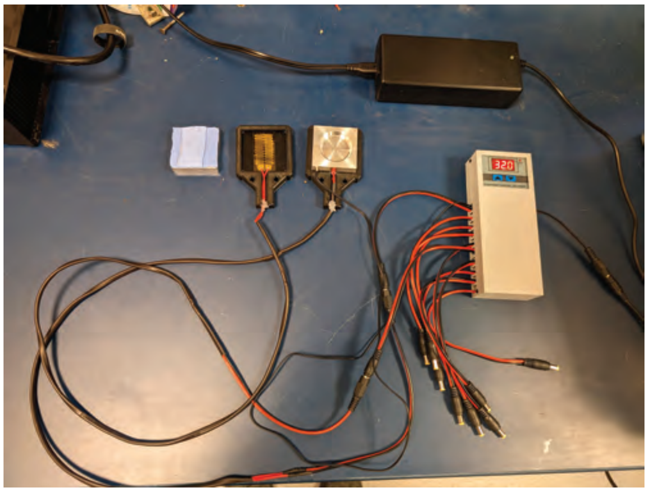

Figure 4.—Modified Salt Lake City Mosquito Abatement District bloodfeeding device. 3D printed base with thin film heater and aluminum blood reservoir (upper left). 3D printed junction box with temperature controller (right).

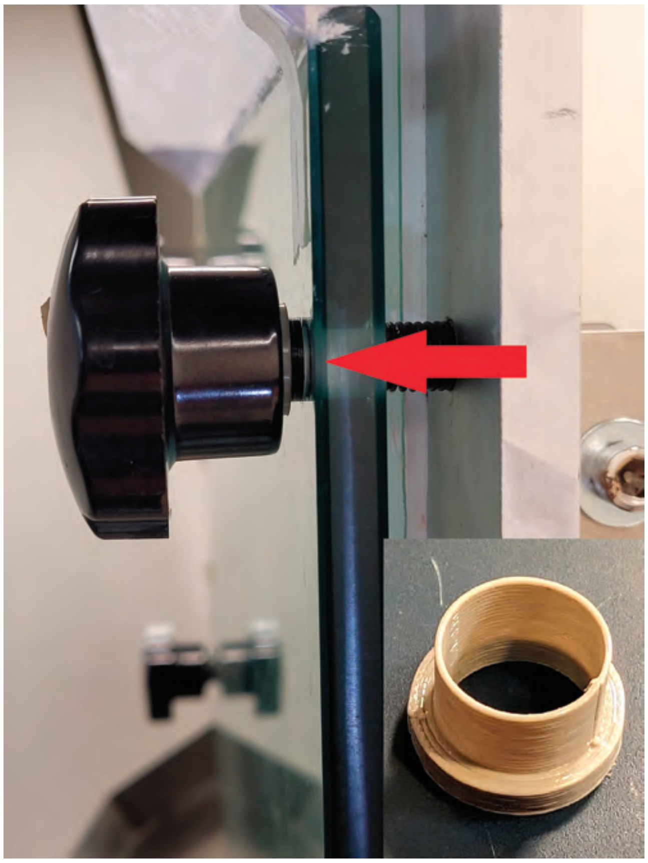

Figure 5.—3D printed bushing for pupal separator installed on pupal separator as well as standalone part (bottom right).

Bushings for the pupal separator (Fig 5) proved very useful. Before adding the bushings, adjusting the pupal separator caused the glass to grind against the bolts producing glass dust. After adding the bushings, the adjustment of the pupal separator was very smooth and no longer damaged the glass.

Conclusion

3D printing presents a promising direction for tailored solutions to address specific challenges encountered in vector control. The wealth of resources online allows districts to easily evaluate different techniques and tools developed by other agencies. Districts can save time and money on the acquisition of specialized parts and tools by 3D printing them quickly and cheaply. Furthermore, 3D printing allows employees to innovate and solve problems that might have gone unsolved due to the rapid prototyping capabilities offered by easy-to-use 3D modeling software.

Acknowledgements

The author would like to thank Mark Nakata from Delta Mosquito and Vector Control District, Ryan Amick from Greater Los Angeles County Vector Control District, Colt Bellman and Timothy Morgan from Orange County Mosquito and Vector Control District, and Gregory White from Salt Lake City Mosquito Abatement District for their support on this project.

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3D Printing: A gateway to budget-friendly solutions at Shasta Mosquito and Vector Control District

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Introduction

Mosquito control is somewhat of a niche industry and subsequently has limited solutions for customizations to make work flows more efficient and efficacious. For decades, Shasta Mosquito and Vector Control District (District) has searched for innovative, efficient, and economical solutions. In an effort to improve our programs, we purchased a 3D printer in 2019, allowing staff to develop solutions across all departments, including the administrative office, aquaculture program, laboratory insectary, mosquito monitoring programs, immature and adult mosquito control programs, and outreach and public education programs.

Methodology

The District purchased a X-Max printer (Qidi Tech, Ruian Economic Development Zone, Ruian, Wenzhou, Zhejiang, China) which offers a printable space of 300mm x 250mm on the build plate and 300mm high in an enclosure (Fig. 1). This was the largest capacity printer available with an enclosure for under $1,500. We upgraded to a compatible high-temperature all-metal hot end from the Qidi company and added a fan and air ducting to accommodate the higher temperatures when printing with applicable filament materials. When designing new solutions, we utilized free web-based software called TinkerCAD (AutoDesk Inc., 160 Greentree Dr Ste 101, Dover, Kent, DE, 19904) which minimized cost and was both easy to navigate and easy for training (Fig. 2). The Qidi X-Max came with its own proprietary slicing software called QidiPrint (Fig. 3), which is the primary slicing software used by our District. We utilized polylactic acid (PLA), polycarbonate (PC), and polyethylene terephthalate glycol (PETG) filaments for various applications (Fig. 4).

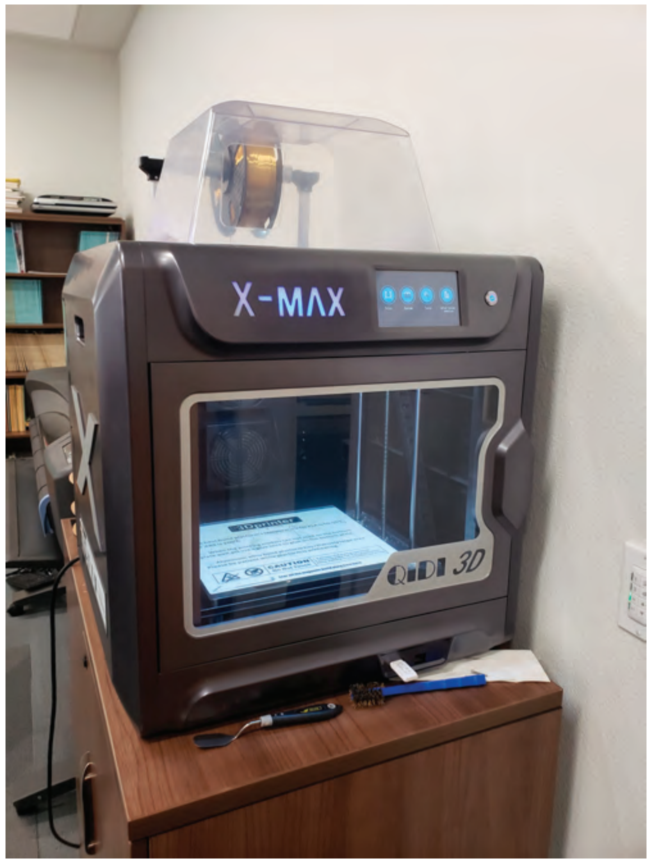

Figure 1.—Qidi X-Max 3D printer.

Results

The District successfully used the 3D printing technology to provide solutions across all departments. Using PLA filament, the District printed indoor display brackets, outreach items, and customized organization tools. Using PC filament, the District printed durable outdoor tools and custom mosquito trap parts. Using PETG filament, the District found an easy solution to some of the difficulties experienced while using PC filament for durable, outdoor items, and at a lower cost.

Figure 2.—Vehicle key tags designed in TinkerCAD web-based software.

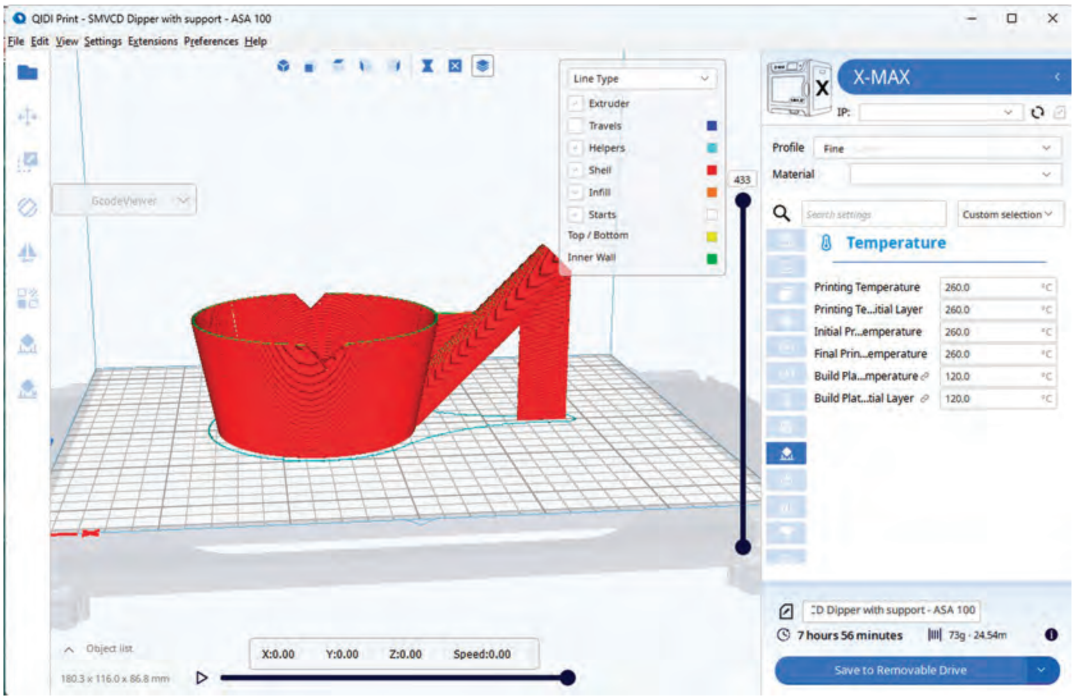

Figure 3.—A dipper sliced in QidiPrint.

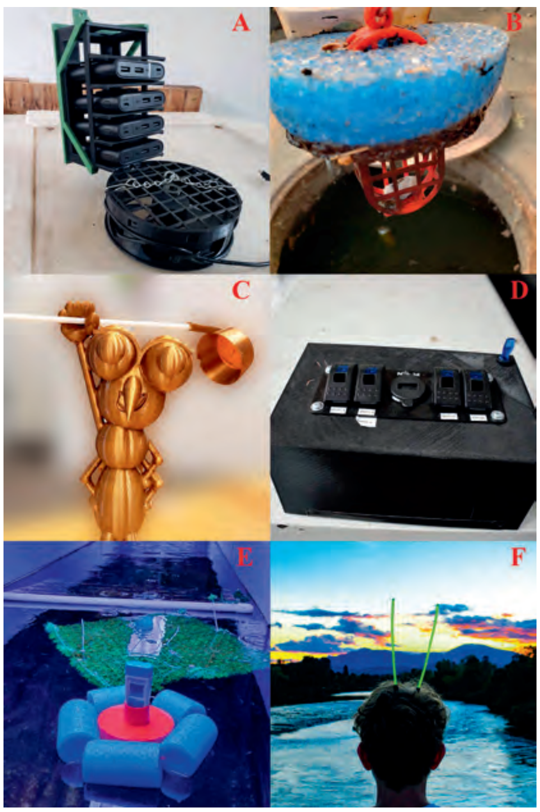

Figure 4.—Examples of 3D printed field applications: (A) trap parts and custom battery charging stations, (B) basket floating larvicide product in heavily organic catch basin, (C) trophy of our District mascot Missy Keeto, (D) box for custom ULV fogger toggle system, (E) water quality monitoring device holder for the aquaculture program, and (F) glow headband at the Lighting of the Sundial Bridge 2020, an outreach event for California Mosquito Awareness Week.

Conclusion

The 3D printing technology allowed us to develop specialty solutions for specific equipment needs across all departments and at a budget-friendly level. Going forward, the District will continue to use PLA for indoor and outreach applications, and will pivot to PETG instead of PC for outdoor and high durability applications.

Acknowledgements

Thanks to District management for continuing to encourage and support the utilization of new technology and experimentation of new, innovative ideas. Many thanks also to the full-time and seasonal staff that have tested the 3D printed items in our District programs. Without field testing and feedback, we would not be able to continue elevating our designs and production. Additionally, thank you to the 3D printing staff in other mosquito districts for feedback and recommendations on filaments and software, all in the spirit of bringing mosquito control in California to a higher level of efficiency.

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TinkerCAD. AutoDesk, Inc. 160 Greentree Dr Ste 101, Dover, Kent, DE, 19904. https://www.tinkercad.com/. 2024**.**

3D Printing at Delta Mosquito and Vector Control District

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Abstract

The increasing accessibility of 3D printing technology allows professionals to fabricate custom components with diverse materials for various applications. In 2020, Delta Mosquito and Vector Control District (DMVCD) acquired a Prusa MK3S+ 3D Printer, modifying it with an aftermarket E3D Revo Six Hotend and an enclosure system equipped with a BlazeCut Fire Suppression System and air filtration system. Autodesk Tinkercad serves as the software program for creating 3D models and designs, while PrusaSlicer is used for slicing these models. The 3D printer has played a useful role in producing custom components for the DMVCD's laboratory, insectary, mosquitofish facility, office spaces, surveillance traps, pesticide field cage trials, and other vector control related equipment. Specifically, objects such as tube racks, 6-inch pesticide disc style field cages, mosquitofish tank modifications, gravid trap parts, EVS traps, BG-Sentinel trap parts, and "squatchy dippers" made from a flexible TPU material have been successfully printed and utilized. The versatility of the 3D printer is evident in its ability to address specific needs using a wide range of different materials. This presentation highlighted the role of 3D printing in overcoming challenges at DMVCD, including the production of parts for discontinued equipment and the realization of innovative ideas.

Barcoding quantitative PCR assay to distinguish between *Aedes aegypti* and *Aedes sierrensis*

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Abstract

Aedes aegypti mosquitoes, known carriers of chikungunya, dengue and Zika viruses, are spreading across California. The ability for vector control agencies to respond quickly to detections of this invasive species is vital to slowing or stopping their propagation. Typical species identification using morphological taxonomy can pose issues for certain specimens, such as atypical and damaged larva and adults, and eggs of the same genus that are morphologically similar. DNA barcoding using the mitochondrial Cytochrome Oxidase I (COI) gene can be used to identify problematic specimens to species given its conserved regions within species and single nucleotide polymorphisms (SNP) that distinguish interspecies differences. In this study, a multiplex quantitative PCR assay was developed to identify ambiguous samples of two morphologically similar species: *Aedes aegypti* and *Aedes sierrensis*. The assay demonstrated an average sensitivity of 97.29% and an average specificity of 94.69% when tested on *Ae. sierrensis*. For *Ae. aegypti*, the assay had an average sensitivity of 98.61% and an average specificity of 93.89%. This *Aedes* identification assay could be employed by vector control organizations to identify potentially invasive *Ae. aegypti* specimens, especially when conventional methods prove inadequate for atypical samples.

Introduction

As *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) mosquitoes continue their spread in California, the risk of mosquito-borne diseases, including chikungunya, dengue, and Zika, may increase (Lwande et al. 2020, Näslund et al. 2021, Simmons et al. 2012). These diseases pose substantial threats to public health, causing fever, joint pain, and in severe cases, neurological complications, and adverse fetal outcomes (Simmons et al. 2012, Vairo et al. 2019, Bertolotti et al. 2020, Petersen et al, 2016). *Aedes aegypti* reproduces principally in water-filled containers that occur around homes (Getachew et al. 2015, Forsyth et al. 2020). *Aedes* (*Ochlerotatus*) *sierrensis* (Ludlow) is broadly distributed in the western United States (Kesavaraju et al. 2014), transmits *Dirofilaria immitis* (heartworm) to dogs (Sacks et al. 2004), and reproduces primarily in the cavities of trees when they contain water (Maciá et al. 2000). The immature forms of *Ae. sierrensis*, eggs and larvae, are morphologically similar to *Ae. aegypti* (Getachew et al. 2015, Champion et al. 2014, Washburn et al. 1993). Oviposition traps, which are containers filled with water and a substrate that passively collect mosquito eggs for monitoring and control, may capture eggs from both *Ae. aegypti* and *Ae. sierrensis* (Lenhart et al. 2005). Detecting where these mosquitoes propagate is crucial for effective control. Accurate identification of vector species is essential because different approaches may be employed to control native and non-native mosquitoes (World Health Organization 2012), especially in response to imported arbovirus cases. Additionally, the earlier the vector can be identified in its life stage, the sooner appropriate control measures can be taken to halt its spread.

The widely used methods of morphological taxonomy to identify eggs, larvae and adult specimens to species have some limitations. Not only is a trained entomological specialist needed to 'key out' a captured specimen, identification using a binomial key and microscope is time consuming and is challenging for badly damaged specimens when distinguishing features are missing or unrecognizable (Beebe 2018, Park et al. 2020). Although differences in eggshell morphology have been found using scanning electron microscopy, these imaging techniques are expensive and may not be available to most vector control operations (Faull et al. 2016, Matsuo et al. 1974). Functionally, *Aedes*-species eggshells do not contain sufficient distinguishing characteristics for vector control technicians to visually identify them to species (de Morais et al. 2019). When morphological taxonomy fails to produce a definitive result, an alternative technique to identify species is needed.

Molecular genetic approaches, such as DNA barcoding, can supplement morphological taxonomy for species identification (Chan et al. 2014, Kumar et al. 2007). The mitochondrial Cytochrome Oxidase subunit I (COI) barcoding region is a useful region for identifying species within the *Aedes* genus, given its conserved regions within species and single nucleotide polymorphisms (SNP) that distinguish species differences (Hebert et al. 2003, Adeniran et al. 2021). To exploit this technique for rapid identification of ambiguous samples to species, we developed a multiplex

Table 1.—Number of mosquitoes tested by species, life stage, and collection site.

Species	Life stage	Collection location			Total (n)
		Alameda (Field-collected)	Orange (Colony)	Sonoma (Colony)	
Aedes aegypti	egg	0	119	0	222
	4th instar larva	0	31	0	
	adult	0	72	0	
Aedes sierrensis	egg	0	0	114	357
	4th instar larva	132	0	24	
	adult	47	0	40	

quantitative PCR (qPCR) assay to distinguish *Ae. aegypti* from *Ae. sierrensis*. Utilizing this *Aedes* identification qPCR (*Ae*ID-qPCR) assay may better enable targeted vector control efforts in regions where *Ae. aegypti* and *Ae. sierrensis* co-occur.

Methods

Mosquito collection

Aedes aegypti were originally collected from Mission Viejo in Orange County, California (strain *Ae. aegypti*-MV) using encephalitis vector survey traps that were baited with dry ice (EVS traps; catalog number 2801A, BioQuip, Rancho Dominguez, CA) and reared in an insectary under standard growth conditions. The laboratory-reared F1 generation *Ae. aegypti* adults and F2 generation eggs and third instar larvae were frozen at −80°C until use. All developmental stages of *Ae. sierrensis* were originally collected from Cotati in Sonoma County, California (strain *Ae. sierr*-C) and reared in an insectary as described above. Additional larval and adult stages were collected in the field from Alameda County in Oakland, California (USA) using EVS traps for adult stages and hand-collected with a larval sampling cup (BioQuip, Rancho Dominguez, CA) for the immature stages. Larval samples from Cotati, California were stored in 70% ethanol prior to testing. The preservation ethanol was removed by pipette and samples were allowed to dry for 15 min before processing. Adult *Ae. sierrensis* were identified species utilizing a Nikon SMZ1000 dissection microscope (Nikon, Tokyo, Japan), chill table (catalog number 1431, BioQuip, Rancho Dominguez, CA) and binomial key (Meyer and Durso 1993). Larval samples from Alameda County were identified morphologically to species. Individual adult and larval mosquitoes were placed in 2 mL microcentrifuge bead mill tubes containing a single 5 mm glass bead (Fisher Scientific, Waltham, MA). Eggs were individually placed into 2 ml microcentrifuge bead mill tubes containing one 5 mm glass bead. Samples were then stored at −80°C until further use. A total of 222 *Ae. aegypti* and 357 *Ae. sierrensis* specimens were assessed using the *Ae*ID-qPCR assay (Table 1).

DNA extraction and quantification

Genomic DNA was isolated from all mosquito life stages using a MagMAX-96 Viral RNA Isolation Kit, which isolates both DNA and RNA (Thermo Fisher Scientific, Waltham, MA) with the Kingfisher Duo Prime Purification System (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer instructions. The concentration of the eluted DNA was quantified using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

qPCR barcoding assay for identifying and evaluating mosquito species

The *Ae*ID-qPCR assay primer and probe sequences that targeted the COI region of *Ae. aegypti* and *Ae. sierrensis* (GenBank No. AF425846.1 and KP293424.1, respectively) were designed using Primerquest software (Table 2; version 2.2.3; Integrated DNA Technologies, Coralville, Iowa USA). MUSCLE multiple sequence alignment tool (https://www.ebi.ac.uk/Tools/msa/muscle/) was utilized to align the two mosquito species sequences and visualize the location of SNPs (Figure 1). The *Ae. aegypti* probe was labeled with ABY with the Iowa Black RQ (IAbRQSp) quencher at the 3'-end and the *Ae. sierrensis* probe with FAM with the IowaBlack FQ (IABkFQ) quencher at the 3'-end.

For the *Ae*ID-qPCR assay, the Primetime Gene Expression Master Mix (Integrated DNA Technologies, Coralville, Iowa) was prepared according to the manufacturer's recommendations, using 5 µl of template DNA (10.5 - 2790 ng)), 900 nM primers, 250 nM probe, 10 µl of the Master Mix, and nuclease-free water to bring to volume of 20 µl. MicroAmp Optical 96-Well Reaction Plate (Thermo Fisher Scientific, Waltham, MA) was vortexed for

Table 2.—Primer and probe sequences that were developed to discriminate between *Ae. aegypti* and *Ae. sierrensis* for the *Ae*ID-qPCR assay.

Name	Sequence (5′ → 3′)
Primers	
*Ae*ID-aegypti-F	TGATTAGCAACTTTACACGGAAC
*Ae*ID-aegypti-R	AGCTAATACTACTCCTGTTAAACCT
*Ae*ID-sierrensis-F	CCTCCTTCATTAACCCTACTACTTT
*Ae*ID-sierrensis-R	GATGATACTCCAGCTAAATGAAGAG
Probes	
*Ae*ID-aegypti-PRB	ABY-TCCAGCCCTTCTATGATCATTAG GATCTGT-IAbRQSp
*Ae*ID-sierrensis-PRB	FAM-TGGAGCTGG/ZEN/TACAGGATG AACTGT-IABkFQ

GenBank Accession Number	Species Name	Sequence	Position
KP293421.1	*Aedes sierrensis*	AGTTTTTGAATATTA**CCTCCTTCATTAACCCTACTACTTT**CAAGATCTATAGTAGAAAAT	354
AF425846.1	*Aedes aegypti*	AGTTTTTGAATACTACCTCCTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAAT	360
KP293421.1	*Aedes sierrensis*	**GGAGCTGGTACAGGATGAACTGT**TTATCCCCCTCTCTCATCAATTCCTGCCCATGCAGGA	414
AF425846.1	*Aedes aegypti*	GGGGCAGGAACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGGA	420
KP293421.1	*Aedes sierrensis*	GCTTCAGTTGATTTAACAATTTTTT**CTCTTCATTTAGCTGGAGTATCAT**CAATTTTAGGA	474
AF425846.1	*Aedes aegypti*	GCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCTCAATTTTAGGG	480
KP293421.1	*Aedes sierrensis*	TTTAGATGATTAGCAACTCTTCATGGAACACAATTAACTTATAGCCCTGCATTACTTTGA	1014
AF425846.1	*Aedes aegypti*	TTTAGT**TGATTAGCAACTTTACACGGAA**CTCAATTAACATATAG**TCCAGCCCTTCTATG**	1020
KP293421.1	*Aedes sierrensis*	TCCTTAGGATTTGTATTCTTATTCACAGTTGGAGGATTAACTGGTGTAGTACTAGCTAAT	1074
AF425846.1	*Aedes aegypti*	**TCATTAGGATCTGT**ATTTTTATTTACAGTTGG**AGGTTTAACAGGAGTAGTATTAGCT**AAT	1080

Figure 1.—Multiple sequence alignment for *Ae. aegypti* and *Ae. sierrensis*. Forward primers are colored in green, probes in yellow, and reverse primers in red.

15 s at maximum speed and centrifuged for 15 s (MPS 1000 Mini PCR Plate Spinner, Labnet International, Inc., Edison, NJ). A QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA) was employed using the Standard Curve setting with the following qPCR cycling conditions: an initial 95°C for 3 min, followed by 40 cycles of 95°C for 5 s and 63.4°C for 30 s. Both baseline and threshold values were automatically determined by the QuantStudio Design and Analysis Software v2.6 (Thermo Fisher Scientific, Waltham, MA).

Identifying species with the *Ae*ID-qPCR assay

The *Ae*ID-qPCR assay was validated by comparing the Ct values with the species of mosquito that was identified morphologically using a microscope as described above or known reference strains (*Ae. aegypti*-O and *Ae. sierr*-S). A correct identification by the *Ae*ID-qPCR assay (i.e., true positive) was defined as when there was agreeement between the visual identification of a specimen and a maximum Ct value of 27.41 for egg samples, Ct of 22.23 for larval samples, and Ct of 16.99 for adult samples with the corresponding probe set.

The following equations from (Altman 2000) were used to determine the average Ct values for a true positive in the *Ae*ID-qPCR assay for each life stage:

Consolidation of combined mean and standard deviation values

$$\sum x = mean \times n \tag{1}$$

$$\sum x^2 = SD^2(n-1) + \frac{\left(\sum x\right)^2}{n} \tag{2}$$

$$tn = sum\ \ of\ \ all\ \ (n) \tag{3}$$

$$tx = sum\ \ of\ \ all\ \sum x \tag{4}$$

$$txx = sum\ \ of\ \ all\ \sum x^2 \tag{5}$$

$$Combined\ \ mean = tx/tn \tag{6}$$

$$Combined\ \ SD = \sqrt{\frac{\frac{txx - tx^2}{tn}}{tn - 1}} \tag{7}$$

The delta RN value (ΔRn) for each qPCR assay was calculated from the difference in the normalized reporter value (Rn) and the baseline signal created by the instrument used. A maximum ΔRn over 1 was used to establish a true positive in conjunction with the maximum Ct values.

The sensitivity and specificity of the *Ae*ID-qPCR assay were calculated using Excel (Microsoft Corporation, Redmond, WA), from the confusion matrices for each sampling region, species, and location (Figure 2) with the equations (Parrikh et al. 2008):

$$Sensitivity = \frac{True\ \ Positive}{True\ \ Positive + False\ \ Negative}$$

$$Specificity = \frac{True\ Negative}{True\ \ Negative + False\ \ Positive}$$

Along with sensitivity and specificity, positive predictive values (PPV) and negative predictive values (NPV) diagnostic measures were used to identify samples to species for the *Ae*ID-qPCR assay. PPV and NPV were calculated from the confusion matrices for each group (Figure 2) using the following equations (Parrikh et al. 2008):

$$PPV = \frac{True\ \ Positive}{True\ \ Positive + False\ \ Positive}$$

$$NPV = \frac{True\ \ Negative}{True\ \ Negative + False\ \ Negative}$$

Confidence intervals (CI) were calculated using the simple asymptotic method without continuity correction and

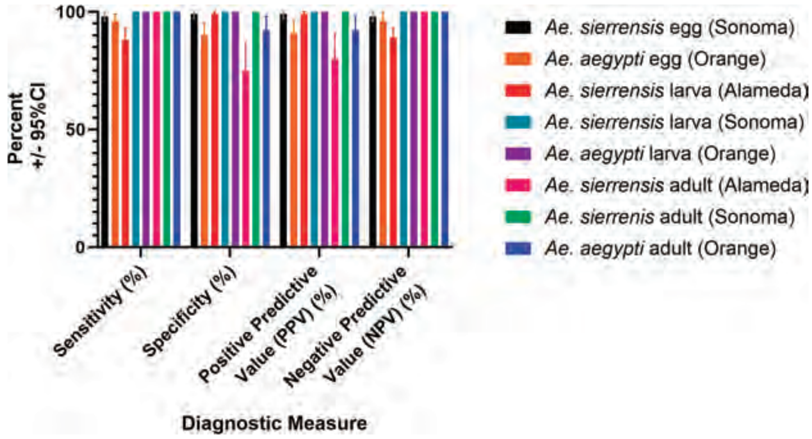

Figure 2.—Accuracy and precision of the qPCR assay according to diagnostic measures with corresponding confidence intervals.

any upper bound of the confidence intervals over 100% were rounded down to 100% (Newcombe 1998):

$$CI = p \pm z\sqrt{\frac{p(1-p)}{n}}$$

Where p is the diagnostic method proportion and z equals 1.96 for 95% confidence.

Results and Discussion

DNA concentrations and Ct values

Aedes sierrensis and *Ae. aegypti* display similar oviposition behavior, produce eggs with similar morphologically (Kesavaraju et al. 2014, Bohart et al. 1978, Zahouli et al. 2016) and have an 88.65% identity (National Center for Biotechnology Information 2023) in the COI gene (KP293421.1, AF425846.1). Although the level of sequence dissimilarity may be sufficient to differentiate between the two species, it may not be optimal for qPCR assay development. Factors such as melting temperature discrepancies and the formation of secondary structures in the nucleic acid can limit the available regions for assay development. A novel primer and probe set was tested on eggs, larvae and adults of *Ae. sierrensis* and *Ae. aegypti* from several locations.

The DNA concentration for adult *Ae. sierrensis* from Sonoma County was 149.3 ± 53.5 ng/μl, larvae was 74.3 ± 32.0 ng/μl, and egg was 13.6 ± 2.8 ng/μl (Figure 3). DNA concentrations for different life stages of *Ae. sierrensis* from Alameda County were similar (adults at 100.4 ± 134.8 ng/μl and larvae at 141.4 ± 76.7 ng/μl). For *Ae. aegypti* from Orange County, DNA concentrations were highest for the larvae (116.8 ± 49.2 ng/μl), followed by adult (75.6 ± 43.6 ng/μl) and egg samples (13.5 ± 13.7 ng/μl). For mosquitoes from Alameda County and Orange County, the mean DNA concentration in larvae exceeded that in eggs. In particular, the mean larval DNA concentration for *Ae. aegypti* from Orange County was 765.2% higher than its egg counterpart. Conversely, mean adult concentrations decreased by 35.3% for *Ae. aegypti* from Orange County and 29.0% for *Ae. sierrensis* from Alameda County when compared to their respective larval stages. For samples from Marin-Sonoma, the mean DNA concentration was highest in adults, exceeding both larvae and eggs. In this set, the mean larval DNA concentration was 446.3% above the egg stage, whereas the adult concentration was 100.9% greater than the larval stage. The primary distinction in the preparation of samples for *Ae. sierrensis* from Alameda and Sonoma county stemmed from how the larval and adult specimens were collected and preserved. Larvae from Sonoma originated from the prior generation of collected adults. The adult samples from Alameda County were stored in a −80°C freezer for up to a year without ethanol, whereas the larvae were gathered just before being tested. The difference in DNA concentrations may be due to the absence of ethanol in the adult specimen tubes prior to their storage at −80°C (Stein et al. 2013). An egg sample that contained a DNA concentration of 2.1 ng/μl was the lowest observed across all eggs, but it successfully amplified in the assay. Overall, Ct values were lowest for adults, intermediate for larvae, and highest for eggs (Figure 4).

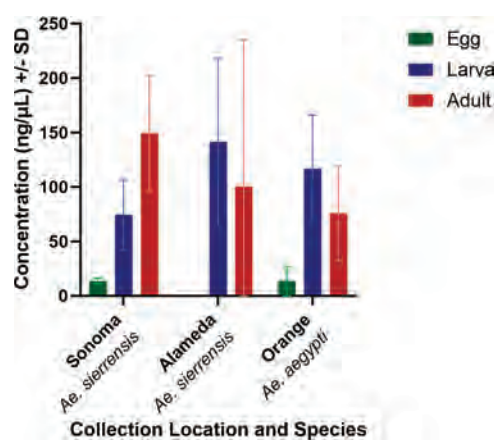

Figure 3.—DNA Concentration pre-amplification (ng/μl) from each location by species and life stage.

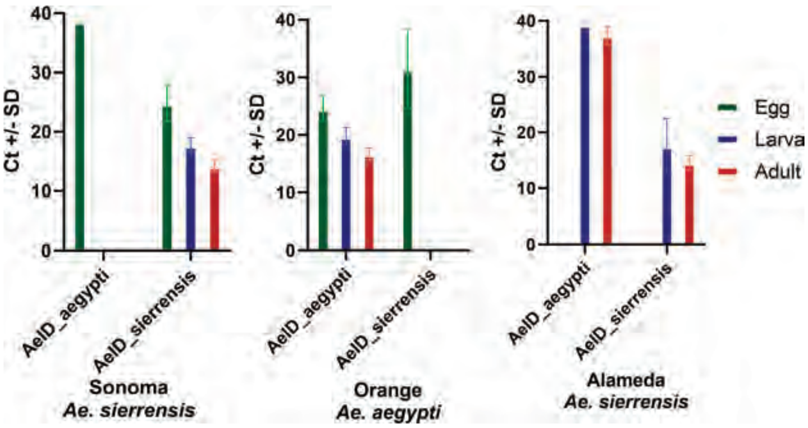

Figure 4.—Mean Ct values for *Ae. aegypti* and *Ae. sierrensis* probes (AeID_aegypti & AeID_sierrensis) across egg, larval and adult life stages and geographic locations.

Because Ct values serve as indicators of the gene copy number, lower Ct values reflect a higher amount of target gene in the sample (Gunay et al. 2016). Therefore, it was anticipated that for the samples from Alameda County and Orange County, larvae would show the highest average Ct values, followed by adults and eggs. The variation in amplification efficiency might stem from the adult mosquito stages having a greater mitochondrial content (Tsang et al. 2002). Because the *Ae*ID-qPCR assay was developed using mitochondrial DNA sequences, differences in the total number of mitochondria across life stages may affect Ct values.

*Ae*ID-qPCR assay amplification plots

Less than 1% of the egg samples of *Ae. sierrensis* (Figure 5) and 10% of *Ae. aegypti* (Figure 6) produced Ct values that indicated a false positive (Table 3). There was a single instance of a false positive in which an *Ae. aegypti* sample amplified the AeID_sierrensis probe and produced a lower Ct than the AeID_aegypti probe (Figure 7). Although this may point to low specificity of the assay, the amplification curve was irregular and displayed a sharp increase in florescence in the early amplification phase (cycle number 3 – 13) and declined shortly after (Figure 7). Atypical amplification curves such as this can occur if the DNA template concentrations are too high (Menezes et al. 2023). For the group, the median concentration was 13.5 ng/µl, but the DNA concentration for this sample was 124.6 ng/µl. In all other cases, when an incorrect probe amplified, the Ct value that was produced from the amplification of the correct probe was lower, indicating the true positive.

For the larval samples of *Ae. sierrensis* from Alameda County, the standard deviation from the mean Ct was the highest at 4.94, indicating greater variation in Ct values relative to the other sample groups. Larval *Ae. sierrensis* had the highest rate of false negatives at 11.8% (Table 3). Despite *Ae. sierrensis* being the most common treehole-breeding mosquito in the western United States (Kesavaraju et al. 2014), other mosquito species may have been present and affected the increased number false negatives for larval *Ae. sierrensis*. There also may have been higher genetic variation as well as size and corresponding DNA concentration in field-collected samples relative to colony-raised *Ae. sierrensis* that contributed to the difference in Ct value standard deviation, 1.244 in colony-raised *Ae. sierrensis* and 4.940 in field-collected *Ae. sierrensis* (S1, S2, S3 Figures).

Identification of mosquito larvae to species can be a difficult process where the presence, length, shape and number of hairs in various locations leads an entomologist to identify a larva to a particular species. Absent or atypical features in a particular specimen could lead to misidentification. Our qPCR assay therefore may be more

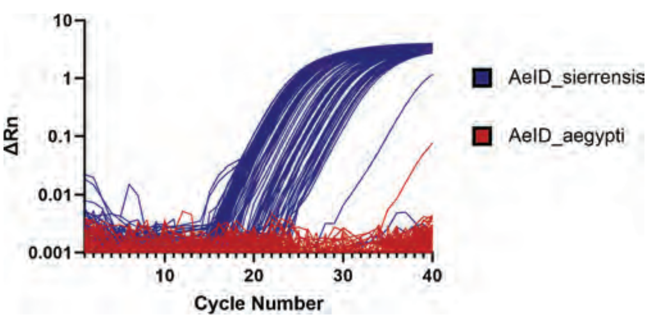

Figure 5.—Amplification plots for each *Ae. sierrensis* egg sample from Sonoma County displaying both probes.

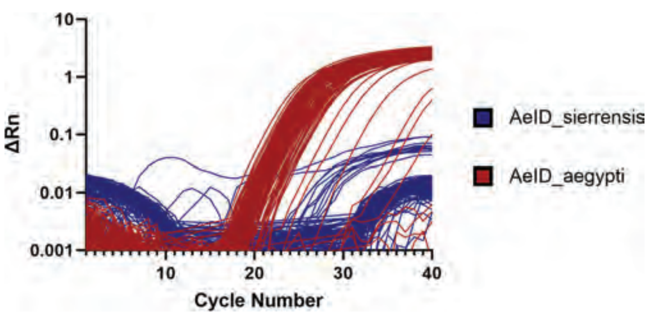

Figure 6.—Amplification plots for each *Ae. aegypti* egg sample from Orange County displaying both probes.

Table 3.—Proportion of incorrect identifications for each group tested by location, species, and life stage.

Location	Sonoma	Orange	Sonoma	Alameda	Orange	Sonoma	Alameda	Orange
Species	*Ae. sierrensis*	*Ae. aegypti*	*Ae. sierrensis*	*Ae. sierrensis*	*Ae. aegypti*	*Ae. sierrensis*	*Ae. sierrensis*	*Ae. aegypti*
Life Stage	egg		larva			adult		
Proportion of False Positives (%)	0.9%	10.0%	0.0%	0.7%	0.0%	0.0%	25.0%	8.3%
Proportion of False Negatives (%)	1.7%	4.2%	0.0%	11.8%	0.0%	0.0%	0.0%	0.0%
n	114	119	24	132	31	40	47	72

likely to provide accurate identification compared to visual morphological taxonomy. On the other hand, adult *Ae. aegypti* samples from Orange County had few false positives, but the Ct values were closer to true positive than adult *Ae. sierrensis* (S4 Figure). In the case of the false positives, the plateau phase never went above a ΔRn value of 0.1. On the other hand, true positives surpassed a ΔRn value of 1. Therefore, when evaluating *Ae. aegypti* adult samples, it's crucial to consider not just the Ct values but also the highest ΔRn value to accurately identify true positives.

Sensitivity and specificity of the *Ae*ID-qPCR assay

The *Ae*ID-qPCR assay demonstrated high sensitivity (Figure 2), with values exceeding 95% for all but *Ae. sierrensis* larvae from Alameda County. These samples had a sensitivity of 88%. High specificity also was found, with most samples scoring above 90%. The exception was field-collected adult *Ae. sierrensis*, with a specificity of 75%. Greater genetic heterogeneity for field-collected mosquitoes in this group may have led to inefficient or non-specific primer or probe annealing to the nucleic acid during PCR.

Positive predictive and negative predictive values

For all sample groups but one, the PPV was greater than 90% (Figure 2), except for adult, field-collected *Ae. sierrensis* samples from Alameda County where the PPV was 80%. Although false positives for this group were higher than others, for every false positive found, a lower Ct with the correct probe was generated in the same multiplex reaction, which pointed to true positives. This highlights the importance of considering the Ct's that are generated by both probes in the multiplex assay rather than a simple examination of target amplification. The negative predictive value was greater than 95%, except for the field-collected larval group from Alameda County that produced an NPV of 89%. If a greater number of mosquito larvae were incorrectly labeled as *Ae. sierrensis*, it would have reduced the negative predictive value for this group. The potential for this misidentification could stem not only from the genetic variety from field collected mosquitoes compared to colony raised mosquitoes, potential PCR inhibition from tannins present in the treeholes (Creer et al. 2010, Mercer et al. 1994), but also from the fact that the species identification of every sample was not verified. Nevertheless, the selective checks did not reveal any other species within this group.

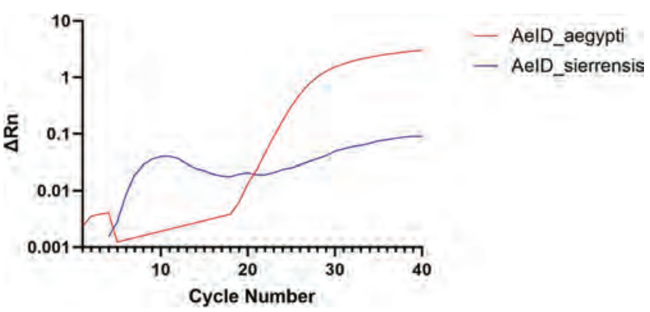

Figure 7.—Single instance of the false positive for an *Ae. aegypti* sample with a lower Ct than the true positive.

Conclusions

Our study highlights the robust capability of this qPCR assay to accurately distinguish between *Ae. aegypti* and *Ae. sierrensis* in the egg and larval stages as well as in instances where adult specimens may be damaged. Although further work could be done to enhance its efficiency, our research validates the assay's effectiveness compared to traditional visual methods. Further investigation is also needed to validate the assay's performance with samples containing mixed specimens. Additionally, the assay's application can be broadened to identify other treehole and container breeding mosquitoes such as *Ae. notoscriptus* and *Ae. albopictus*, although this may require different barcoding regions to distinguish closely related species accurately. The method's utility extends beyond individual specimens; it also shows promise for environmental DNA (eDNA) analysis, offering a practical tool for identifying mosquito species in their natural habitats such as tree holes or yard containers even in the absence of physical specimens. Accurate identification is vital for implementing effective mosquito control measures and for appropriately informing the public about potential disease risks. Therefore, this qPCR assay serves as a useful advancement in evolving our current and future mosquito surveillance efforts.

Supplemental Material

S1 Figure. Amplification plots for each *Ae. sierrensis* larva sample from Sonoma County displaying both probes.

S2 Figure. Amplification plots for each *Ae. sierrensis* larva sample from Alameda County displaying both probes.

S3 Figure. Amplification plots for each *Ae. aegypti* adult sample from Orange County displaying both probes.

S4 Figure. Amplification Plots for each *Ae. sierrensis* adult sample from Alameda County displaying both probes.

Acknowledgements

We would like to express gratitude to Sokanary Sun from the Orange County Mosquito and Vector Control District for providing all *Aedes aegypti* samples, as well as Kristen Holt from the Marin/Sonoma Mosquito and Vector Control District for providing many *Aedes sierrensis* samples. No permits were needed for any specimens transported outside of their normal geographic range because these samples were shipped frozen or preserved.

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Methods to determine *Aedes aegypti* age structure in the Los Angeles Basin

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Introduction

Although the likelihood of pathogen transmission is much higher at sites where mosquito age structure skews older (Ernst et al. 2017), the lack of an affordable, convenient and replicable method for age grading *Aedes aegypti* females makes it difficult for laboratories and vector control districts to study the impact of their interventions on the age structure of wild populations. In this presentation, we discussed the current methods of age grading used in the field and the reasons why each is often not suitable for everyday vector control studies. We then discussed two promising emerging methods of spectroscopic age grading for mosquitoes, which are near-infrared spectroscopy (NIRS) (Chen et al. 2022) and Raman spectroscopy (Gao et al. 2023), and presented our current experimental design for testing the reliability of these methods.

Methods

During the summer of 2023, we collected approximately 2,500 individual *Ae. aegypti* with location data from sites around the Los Angeles Basin area, using BG Sentinel, EVS, and Reiter-Cummings Gravid traps. Mosquitoes were frozen at −70C and shipped to the DART laboratory, where they have been grouped by the climate at their site of origin. A genetically similar control group of Los Angeles Basin *Ae. aegypti* are being reared and will be used to establish controls for age grading. Our study is designed to observe the effects of climate and genetics on spectroscopic methods of age grading to determine if they are a universalizable tool for the age grading of wild mosquitoes.

Results and Discussion

Since this experiment is currently still ongoing, its final results will be submitted to the 2025 edition of the MVCAC proceedings. A diagram of the currently intended approach is included in Figure 1. The eggs collected were used to rear an initial generation (F0) of *Ae aegypti* in our insectary and their offspring (F1) will be aged for 28 days, with sample females removed and individually frozen at −80C on days 1, 3, 7, 14, 21, and 28. To control for metabolic changes after a blood meal, separate cages will contain oviposition papers for blood-fed and twice-blood-fed females, which will be removed and individually frozen on days 7 and 14. These mosquitoes will be used as controls for Raman and NIRS age grading models.

Because near-infrared spectroscopy is nondestructive and only requires the mosquito cuticle, whereas Raman spectroscopy is destructive but only sensitive to changes in chemical composition, we can use the same individual wild mosquitoes to test multiple methods of age grading. Parity determination is likely to damage the cuticle, but unlikely to affect Raman spectroscopy, so one group of samples will be dissected for parity determination and then full-body Raman spectroscopy. Removing the wings and legs is unlikely to damage the cuticle significantly, so the other group of samples will undergo partial Raman spectroscopy of the legs and wings while the cuticles are sent to our partners at the Riehle Laboratory within the University of Arizona for near-infrared spectroscopy analysis.

Conclusions

Spectroscopic methods appear to be promising tools for age grading wild *Ae. aegypti* populations, and research is still underway to determine if these methods are applicable across a range of climates and mosquito genotypes. Our work is attempting to determine the effectiveness of these spectroscopic approaches under varying environmental and genetic conditions. It appears likely that spectroscopic methods of age grading can serve as a relatively inexpensive method of tracking mosquito age structure, but further study is necessary to confirm this.

Acknowledgements

Thanks to everyone at GLACVCD for being instrumental in helping harvest the mosquito samples for this experiment, and to Olivia Winokur and Cynthia DuBridge for their encouragement and assistance in presentation design and delivery.

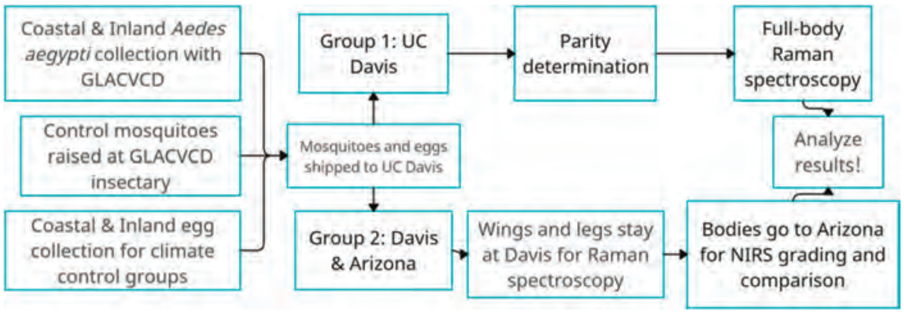

Figure 1.—The fate of *Aedes aegypti* collected from the Los Angeles Basin. Two groups of mosquitoes from the same habitats will be used to compare spectroscopic methods of age grading for wild *Ae. aegypti* with two control groups.

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Exclusion of *Aedes aegypti* from yard drains reduces adult abundance in Madera, California

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Abstract

The invasive mosquito, *Aedes aegypti,* poses health risks in urban areas due to its ability to transmit dengue and several other arboviruses, its preference for blood-feeding on humans, and its prolific production in human-made containers found around homes. Our study in Madera, California determined whether blocking mosquito access to yard drains with both a cap and plastic matrix could reduce adult *Ae. aegypti* abundance in a single suburban neighborhood. The 10-week study followed a stepped-wedge design with three phases. Forty-five Biogents Sentinel traps were placed in the targeted area and monitored daily. For the first two weeks, monitoring occurred without any intervention to establish baseline data. Starting from week 3, houses in the northern wedge had their lawn drains capped, followed by the southern wedge in week 5 and the central wedge in week 7. Our findings revealed a substantial reduction in the *Ae. aegypti* population within the study area. This research highlights the practical importance of managing *Ae. aegypti* populations through source reduction in an urban community and its potential impact on public health.

From Common to Cryptic: Finding *Aedes aegypti* sources in the Coachella Valley

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Introduction

The *Aedes aegypti*, an invasive mosquito known to transmit exotic viruses to humans, including dengue, chikungunya, yellow fever and Zika, has been introduced into California and was first detected in the Coachella Valley in May 2016. This species exploits artificial and natural containers for larval development. The following is an overview of our program that was designed to be efficient and effective for day-to-day operations.

Methods

Certified Vector Control Technicians (VCT) conduct surveillance regularly throughout the Valley and are on the lookout for invasive mosquito species. The District's Call Center staff are trained to ask questions of residents complaining of mosquito bites about the time of day the mosquitoes are biting and the size and color of the mosquitoes. VCT are responsible for responding to service requests assigned to them. If the presence of mosquitoes is confirmed by the VCT at the residence of the requestor, the technician will inspect the property. During the property inspection, the VCT will focus on educating the residents in ways to prevent mosquito breeding on their property as well as performing physical control as necessary based on the results of the inspection. The task may be time-consuming, depending on the size of the property and the number of potential sources, such as shown in Figure 1. Fountains, flowerpots, yard drains and saucers are common sources for this species (Figure 2). Fountains may be mistaken for sculptures or statues, causing technicians to overlook them as potential larval sources. In addition, fountains may have hidden reservoirs, which may go unnoticed if covered with dirt, gravel or vegetation (Figure 3). A thorough inspection is crucial to ensure all potential sources are discovered. A

Figure 1.—Residential property with multiple potential sources.

Figure 2.—Flowerpots commonly retain water and are common sources for *Ae. aegypti*.

Figure 3.—Fountain with a hidden basal water reservoir.

rigorous inspection helped us identify cryptic sources such as shown in Figure 4. Cryptic sources may not be obvious and may be difficult to access, but they are crucial to our efforts to control this species. Examples of other cryptic sources include tree holes, utility boxes and children's toys that can hold even a small amount of water. Our approach to controlling *Ae. aegypti* is environmentally sound. We locate and dispose of common and cryptic sources to benefit our residents and the environment.

During August 2023, Hurricane Hillary produced an extraordinary amount of rainfall which averaged 3.5 inches across the Coachella Valley and caused a marked increase in the *Ae. aegypti* population developing in storm debris (Figure 5). As a result, service requests increased by 23% during August to December 2023 compared to the previous year. Each of the two full-time technicians and four seasonal staff completed an average of 5 to 7 service requests per day to address the surge. Additionally, the public outreach department took part in city events to raise awareness. Furthermore, the technicians educated

Figure 4.—Tree stump with cryptic source.

Figure 5.—Debris left after Hurricane Hillary.

the public about ways to reduce or eliminate sources. The increase in the mosquito population posed a potential public health risk and biting nuisance, and our team worked diligently to maintain a safe and healthy environment for all residents of the Coachella Valley.

Results and Discussion

The goal of District operations is to reduce mosquito populations and the risk of virus activity through the implementation of best-integrated vector management practices. We collaborate routinely with our residents, public outreach, and laboratory staff to conduct follow-up inspections. When dealing with *Ae. aegypti*, it is imperative to conduct comprehensive inspections to identify potential larval sources in urban landscapes. As a result, we are making progress toward fulfilling the district's mission of improving the quality of life in our communities.

Conclusion

Our surveillance and treatment efforts have shown the challenges of *Ae. aegypti* control. We plan to continue using best-integrated vector management practices to help mitigate our *Ae. aegypti* problem. As new innovations in control become available, we will utilize these resources to evolve our program.

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To lure or not to lure: a comparative analysis of BG sentinel and modified carbon dioxide traps for *Aedes aegypti* surveillance

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Abstract

Mosquito surveillance is a core component of integrated vector management programs and represents the foundation of vector control strategies. Biogents sentinel traps are currently considered the 'gold standard' for *Aedes aegypti* surveillance; however, these traps can be costly to purchase and cumbersome to operate. The current study compared the efficacy of a modified carbon dioxide (CO_2) baited trap with a BG sentinel trap (BG-S) with and without BG lure over the course of several weeks at eight sites in Sacramento. *Aedes aegypti* total catch numbers were analyzed to identify abundance trends and overall differences between trap types. The BG-S captured significantly more *Ae. aegypti* mosquitoes than both the modified and unmodified CO_2 trap. There were no differences between CO_2-baited BG-S traps with or without the BG lure. As invasive *Aedes* species continue to disperse throughout Sacramento and Yolo Counties, our urban surveillance efforts should shift to new and innovative approaches that are less intrusive for residents and equally efficacious in helping fight the bite.

Introduction

The anthropophagic mosquito, *Ae. aegypti aegypti*, is a competent vector of yellow fever (YFV), Zika (ZIKV), chikungunya (CHIKV) and dengue (DENV) viruses (Lounibos and Munstermann, 1981; Powell and Tabachnick, 2013). Since its arrival to the United States during the 17th century, the yellow fever mosquito rapidly disseminated across the country in conjunction with the expansion of human populations. The first detection in California occurred in 2013 in the southern portion of the State (Gloria-Soria et al., 2014; Porse et al., 2015; Pless et al., 2017) and by 2019, *Ae. aegypti* was detected in Sacramento County and has now spread to 28 out of the 58 counties in the State, putting public health and vector control agencies on high alert (CDPH, 2024). Historically, travel-associated dengue cases represent the greatest disease risk associated with invasive *Aedes* in California (CDPH, 2024); however, in 2023, the first two, locally acquired dengue fever cases were reported in Southern part of the state (CDPH, 2023), introducing the possibility for this arbovirus to produce outbreaks and expand throughout the region. Therefore, public health agencies and mosquito abatement districts have established a proactive approach by implementing surveillance measures to monitor population abundance and virus infection in this invasive species. Presently, a variety of surveillance tools are utilized in the United States for surveillance of this invasive species including oviposition traps (ovitrap), BG-S (Biogents, Regensburg, Germany), CDC miniature light traps, etc., all of which exploit the ecology and behavior of *Ae. aegypti* (McGregor and Connelly 2021).

Because *Ae. aegypti* utilize artificial containers in urban environments as developmental sites for progeny, the ovitrap serves as a tool to target females looking to oviposit whereas the BG-S exploits the host-seeking behavior of *Ae. aegypti* by incorporating a BG-Lure (Biogents, Regensburg, Germany), consisting of ammonia, lactic acid, and caproic acid, designed to mimic the attractiveness of human skin scent (Amos et al. 2020; Christophers 1960). Several modifications and new strategies have been developed to the original trap models, which have enhanced overall trap efficacy. For example, the addition of an autocidal, such as pyriproxyfen, to the ovitraps added a control strategy layer to *Ae. aegypti* surveillance (McGregor and Connelly 2021). The addition of carbon dioxide to BG-S traps, in conjunction with BG-lures has been shown to enhance the overall trap efficacy while simultaneously targeting urban *Culex* populations (Wilke et al. 2019).

Currently, Sacramento Yolo Mosquito and Vector Control District (SYMVCD) utilizes BG-S traps, the 'gold standard' for *Ae. aegypti* urban surveillance (Unlu and Baker, 2018; Wilke et al., 2022), to monitor abundance and direct Integrated Vector Management (IVM) practices of District's Urban Operations Program in Sacramento and Yolo counties. However, despite the efficacy of these traps, several drawbacks potentially limit their operational deployment, including substantial trap cost as well as their cumbersome size, weight and additional trapping equipment such as batteries, catch bags and cone nets. Additionally, BG-S traps also add to total trap deployment expenses.

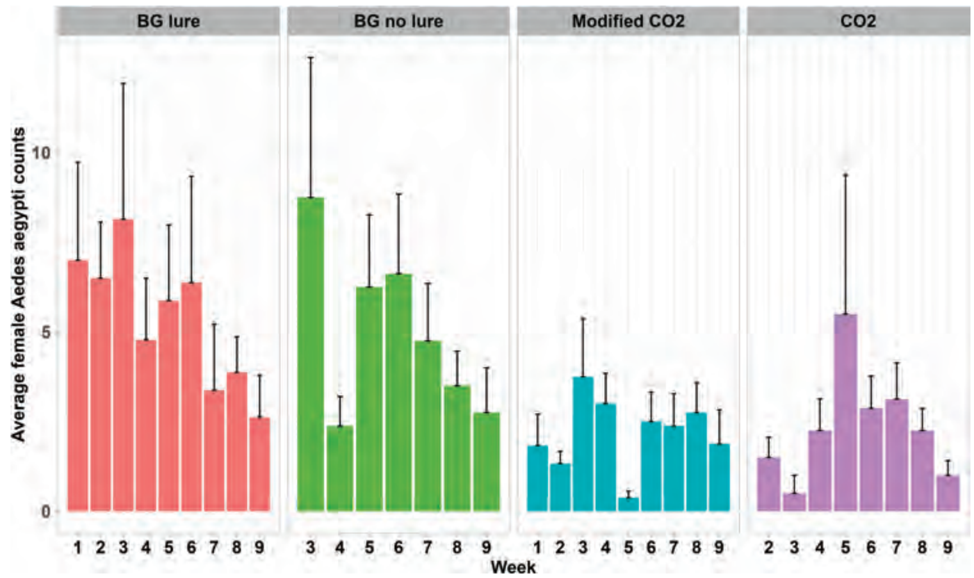

Figure 1.—Average weekly female *Aedes aegypti* catch rates per trap-day between trap types. Bars represent the standard error of the mean.

Overall, these operational issues prompted the need to evaluate alternative surveillance tools that are easily deployable as well as cost-effective. One alternative could be using more compact and economical mosquito surveillance traps such as the CO_2 trap currently used for *Culex* surveillance. In this project, we compared the ability of CO_2 traps, unmodified and modified with a BG lure (Biogents, Regensburg, Germany), and BG-S traps, with and without a BG lure, to collect *Ae. aegypti* and determine if CO_2 traps could be used as an effective surveillance alternative for evaluating invasive *Aedes* populations.

Methods

The study was conducted from 11 Sept to 6 Nov 2023, in the Northgate/South Natomas areas of Sacramento County. Although the *Ae. aegypti* population in this area had only been established in 2022, this is one of the most active infestations in the County. Eight sites were selected based on 2022 and 2023 surveillance data. The two trap types utilized for the study were the BG-S and CO_2 traps. In total, there were four trap permutations: 1) a BG-S baited with a BG lure and dry ice, 2) a BG-S baited with dry ice, 3) a standard CO_2 trap baited with dry ice, and 4) a modified CO_2 trap baited with dry ice and a BG lure. To limit potential location bias, a randomizartion generator was utilized to establish two groups, A and B, at each with four sites, which would determine the trap type each group would receive first. This was done to enable simultaneous co-occurring treatments and maximize the number of replicates per trap type. Specifically, group A started trapping every week with BG-S baited with dry ice and a BG lure, and group B started trapping every week with modified CO_2 traps baited with dry ice and a BG lure. Each group consisted of four sites and each trap type was rotated the following day until all four trap permutations were deployed throughout the week. In total, we had eight replicates per trap type with eight traps. The traps were set at 10:00 am and collected the following day around the same time, letting each trap run for 24 hrs.

Traps were set at the same location in the front yard for the duration of the study, and placed near potential developmental sources in shaded areas, ensuring that the BG-S were placed as close as possible to the location of where the suspended CO_2 traps were hung. Additionally, when hanging the lure for the modified CO_2 trap, fishing wire was used to attach the lure to the handle of the dry ice container and suspend it as close as possible to the motor unit to mimic the circulation exhibited by a BG-S. Both BG-S trap permutations were deployed with a 12V Powersonic battery, whereas the motor units for the CO_2 trap permutations used three, 1.5V D-Cell batteries.

Data Analysis

All statistical analysis used R v4.2.1 (R Core Team 2022). We fitted a generalized linear mixed model (GLMM) using the lme4 package (Bates et al., 2015) to assess *Ae. aegypti* male and female catch between different trap types, considering the influence of temperature, humidity, date, and location. We fitted several models that included or excluded different parameters and interactions and selected the model that had the lowest AIC value (1048). A Wald test using the emmeans package (Lenth, 2024) was used for post-hoc analysis among trap catches.

Results

For female *Ae. aegypti* (Figure 1), the daily average number captured by the BG-S with lure was 5.4 females per trap-day compared to 4.7 by the BG-S without lure. There was no significant difference (Estimate = 0.08, SE = 0.18, z = 0.48, p = 0.96) in the overall catch rates

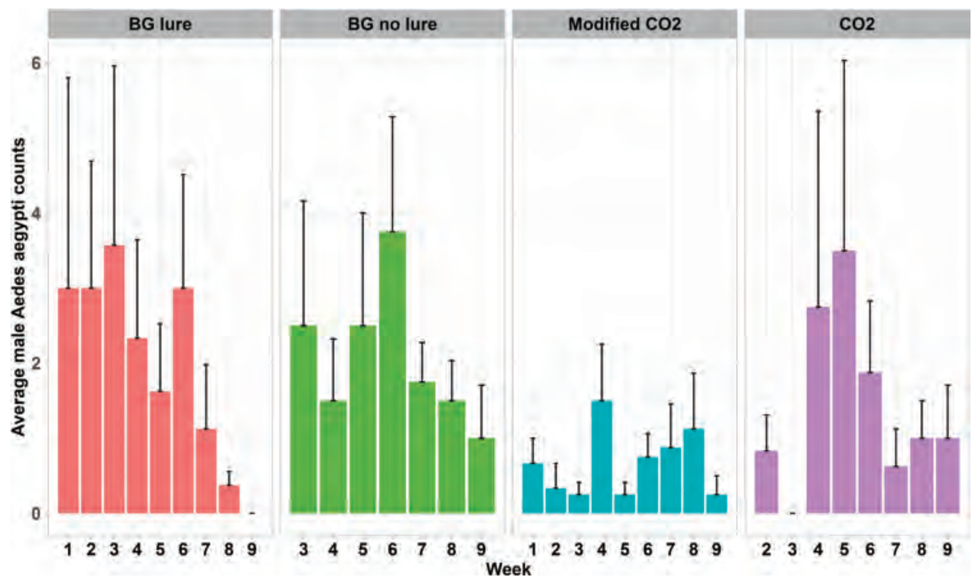

Figure 2.—Average weekly male *Aedes aegypti* catch rates per trap-day between trap types. Bars represent the standard error of the mean.

between trap types. However, the BG-S trap with lure captured a significantly more female *Ae. aegypti* compared to both the modified CO_2 trap (2.2 females per trap day, estimate = 0.98, SE = 0.10, z = 9.5, $p < 0.0001$) and standard CO_2 trap (2.2 females per trap day: estimate = 0.82, SE = 0.18, z = 4.37, $p < 0.005$). Additionally, the BG-S without lure also showed significantly higher capture rates than both the modified CO_2 trap (Estimate = 0.89, SE = 0.19, z = 4.6, $p < 0.0001$) and standard CO_2 trap (estimate = 0.74, SE = 0.12, z = 6, $p < 0.0001$). No significant differences in capture rates were observed between the modified CO_2 trap and the standard CO_2 trap (estimate = −0.15, SE = 0.2, z = −0.7, p = 0.84) Overall, temperature showed no statistically significant effect on catch rates (estimate = −0.09, SE = 0.0593, z = −1.641, p = 0.10). However, there was a significant positive association between the modified CO_2 trap and temperature (estimate = 0.29, SE = 0.017, z = 17.267, $p < 0.0001$). Conversely, humidity had a significant negative relationship with female *Ae. aegypti* capture (estimate = −0.18, SE = 0.07, z = −2.3, p = 0.01). This effect was consistent across all trap types

The average number of male *Ae. aegypti* per trap day (Figures 1 and 2) was lower than females. For both the BG-S with and without lure, the daily average number of captured males was two per trap day and these means were not significantly different (estimate = −0.32, SE = 0.29, z = −1 p = 0.70). However, the BG-S with lure captured a significantly greater number of mosquitoes compared to the modified CO_2 trap (0.6 males per trap day, Estimate = 1, SE = 0.19, z = 5.5, $p < 0.001$) but not the standard CO_2 trap (1.46 males per trap day, Estimate = 0.18, SE = 0.304, z = 0.59, p = 0.93). Interestingly, the BG-S without lure showed significantly greater capture rates than both the modified CO_2 trap (estimate = 1.39, SE = 0.31, z = 4.3, $p < 0.0001$) and standard CO_2 trap (estimate = 0.5, SE = 0.18, z = 2.78, $p < 0.05$). There was a significant negative interaction between temperature (estimate = −0.35, SE = 0.07, z = −4.915, $p < 0.0001$) and humidity (estimate = −0.56, SE = 0.10, z = −5.5, $p < 0.0001$) and the different trap types, suggesting that increases in either factor correlated to reduced captures. Mosquito catch also was influenced by location and trapping dates.

Discussion

Overall, both permutations of the BG-S (with and without lure) were significantly more effective at capturing female *Ae. aegypti* compared to both permutations of the CO_2 traps. However, when analyzing the results for male *Ae. aegypti* capture, both permutations of the BG-S and the standard CO_2 trap were statistically more efficacious in comparison to the modified CO_2 traps. In part, one factor that may have contributed to these results was the difference in voltage and suction capacity between trap types. BG-S traps are powered by a 12V battery in comparison to the CO_2 traps which are powered by three 1.5V batteries, therefore an increase in power output could potentially lead to higher fan speeds, which in turn could have a positive correlation with how well the attractant(s) were circulated throughout the environment. Likewise, this difference in voltage also could impact the suction and retention capability of *Ae. aegypti* that were attracted to the trap.

Two of the most important abiotic factors that impact the holometabolous life cycle of all mosquito species are temperature and humidity (Drakou et al., 2020; Brown et al., 2023). Because mosquitoes are poikilotherms, fluctuations in ambient temperature regimes can induce heat stress, which can impact their survival, distribution, host-seeking, blood-feeding, flight activity, and vector competence (Brady et al. 2013; Nik Abdull Halim et al. 2022; Reinhold et al. 2018; Upshur et al. 2019; Christophers 1960). Likewise, in

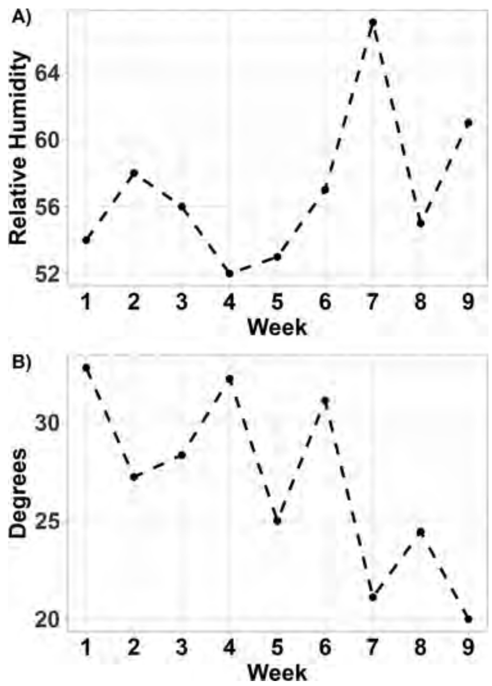

Figure 3.—Average weekly A) temperature (° C) and B) humidity (%) measured at the Sacramento International Airport weather station. Weekly measurements represent the average high from a single reading per week.

conjunction with changes in temperature, fluctuations in humidity can impact survival as well as oviposition patterns and egg production, which could correlate with reductions in population abundance (de Almeida Costa et al. 2010). However, despite the impacts that both of these abiotic factors are known to have on mosquito survival and metabolic activity, for our trial both the temperature and humidity were fixed parameters and not specific to each individual trap site (Figure 3). Instead, all temperature and humidity readings were recorded from the Sacramento International Airport weather station, therefore, we cannot conclusively state that the results from our analysis truly affected catch rates between traps at each individual trap site as well as concretely attribute these factors to variation in abundance (Figure 3).

IVM practices including insecticide applications, in the form of larvicides and adulticides, as well as cultural control measures, in the form of harborage and developmental source reduction play an integral role in population management and control of *Ae. aegypti* (Dwyer et al., 2016; Forsyth et al., 2020). During the course of the study, the District's Urban Operations Program performed wide-area larvicide spraying (WALS), during week five of the study, which could have impacted the trap collection rates for both male and female *Ae. aegypti* (Figures 1 and 2). Additionally, several localized adulticide and larvicide treatments occurred at each of the surveillance sites and neighboring residential properties, which may also have disrupted population dynamics (Garcia-Luna et al., 2019; Morgan et al., 2021; Williams et al., 2022). Lastly, another factor that could have impacted adult detections was harborage and developmental source availability (Getachew et al., 2015; Trewin et al., 2021). During weeks four and seven of the study, several of the trapping sites conducted brush cutting around the property, significantly reducing the amount of vegetation available for mosquito harborage. Additionally, some residents and control technicians drained developmental sources at the study sites and surrounding homes, which may have contributed to fluctuations in mosquito abundance during the course of the study.

Conclusion

Overall, the goal of the study was to conduct a comparative analysis between two BG-S trap and two CO_2 trap permutations at eight sites in the Northgate/South Natomas area of Sacramento County, California. Trap efficacy was based on *Ae. aegypti* collections over the course of nine weeks. Although BG-S, baited with dry ice and a BG lure, remain the industry gold standard, this study demonstrated that BG-S without a lure are comparable for *Ae. aegypti* surveillance. In addition, the standard CO_2 trap demonstrated promising potential as an *Ae. aegypti* surveillance tool, in this geographic location, but should still be utilized in conjunction with BG-S for a more representative measurement of invasive mosquito population dynamics and disease risk potential. Finally, although this study focused on invasive *Ae. aegypti*, all four trap permutations were also effective in capturing other vector species of public health significance, such as *Culex pipiens* and *Culex tarsalis*, which has enabled the District, to utilize this by-catch for evaluating WNV risk in residential neighborhoods. Collectively, these efforts emphasized the role that surveillance and surveillance tools play in directing urban IVM programs and assisting in our knowledge and understanding of population dynamics to guide subsequent control efforts.

As *Ae. aegypti* populations continue to expand their geographic range and distribution in Sacramento and Yolo Counties, surveillance must continue to serve as the guiding principle for urban IVM practices. Additionally, these efforts should not only continue for *Ae. aegypti* but also for *Ae. albopictus* populations, which were first detected in Sacramento County in 2022. Trends should be noted in future studies evaluating trap preference between both species, especially in areas where both species co-occur. Likewise, comparative analysis between these four trap types should continue to be evaluated in all known areas of infestation during the May to October *Ae. aegypti* season. Lastly, a comparative analysis should be conducted analyzing abundance variations in trapping in the front yard versus the backyard of residential homes to determine where residents most frequently encounter these vectors of public health significance.

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Development and field testing of attractive toxic sugar bait station designs for control of *Culex quinquefasciatus* (Diptera: Culicidae) within underground storm drainage systems in the Coachella Valley of Southern California

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Abstract

Attractive toxic sugar bait (ATSB) stations were designed and tested under field conditions for control of mosquitoes (Diptera: Culicidae) within underground storm drain systems (USDS) in the Coachella Valley of southern California. During 11 seasonal experiments over five years, ATSB formulations containing 1% boric acid toxicant were used with a variety of bait station designs and deployment strategies to encourage feeding on the ATSB by adult mosquitoes within the USDS. Effectiveness of ATSB deployment to reduce adult or immature mosquito numbers was compared for USDS chambers containing ATSB stations relative to USDS chambers containing bait stations without toxin (ASB-T) and/or USDS chambers without bait stations (no-bait). Adult mosquito abundance within USDS chambers, and occasionally also aboveground, was monitored weekly using CDC-style UV light traps before, during, and after each bait station deployment period. Immature mosquito abundance within USDS chambers containing standing water was monitored using a mosquito dipper and occasionally also with plastic tubs containing attractive ovipositional media (ovitubs). Sugar baits were colored with green or red food coloring and adult mosquito feeding on the provided baits (ATSB or ASB-T) was evaluated by the proportion of mosquitoes captured with evidence of bait color in the crop or abdomen. When considered over all seasonal trials, ATSB treatments were ineffective for reducing numbers of adult and immature mosquitoes within the USDS. Relatively few adult mosquitoes appeared to feed on the ATSB with $<$ 1% of adults captured in UV traps showing evidence of bait feeding. Implications of this research on the use of ATSB stations for control of mosquitoes within USDS are discussed.

Introduction

Underground storm drain systems (USDS) typically comprise many miles of horizontal drain channels connecting numerous catch basins and manhole chambers serving municipalities to remove runoff from various sources (Kluh et al. 2001, Su et al. 2003). Mosquito abatement districts face a daunting task to control mosquito production within USDS due to abundant difficult-to-access sites that can provide an opportunity for immature development and adult harborage. In southern California, these systems produce an abundance of *Culex quinquefasciatus* Say (Mulligan and Schaefer 1982, Kluh et al. 2006) and therefore increase risk for transmission of arboviruses such as West Nile virus in the surrounding urban area. Attempts to reduce vector production within USDS have had variable success, with methods tested including the application of oils (Pfuntner 1978), insecticides including chlorinated hydrocarbons, organophosphates, and pyrethroids (Kluh et al. 2006), biorational control agents including predatory mosquitofish (Mulligan et al. 1983), insect growth regulators (IGRs) (Stewart 1977), or entomopathogenic bacteria including *Bacillus thuringiensis* subsp. *israelensis* Berliner (Bacillales: Bacillaceae) and *Lysinibacillus sphaericus* (Meyer and Neide) (Bacillales: Bacillaceae) (Mulligan et al. 1981, Kluh et al. 2001). Additionally, physical options have been applied including sea water flushes (Schoeppner 1977) and structural retrofitting (Mulligan and Schaefer 1982). Treatment of USDS to reduce mosquito production can be costly due to additional personnel hours to identify and treat production sites repeatedly each season. Treatments may fail as horizontal trunk lines between human-accessible USDS openings provide opportunities for adult mosquitoes to avoid treatments. Furthermore, many insecticides are prohibited from application at these key mosquito production sites due to environmental regulations intended to protect the underground water supply. These and other factors can hinder sustained success of mosquito abatement efforts in the USDS environment (Kluh et al. 2006).

Attractive toxic sugar bait (ATSB) is an alternative to traditional mosquito control within USDS. Sugars are an essential dietary component for adult mosquitoes of both sexes, and when combined with insecticides can target and kill adult mosquitoes. Use of ATSB in mosquito abatement strategies has been long proposed but only recently implemented in field trials (Lea 1965, Foster 1995, Xue et al. 2006, Fulcher et al. 2014). ATSB use attractive odors such as from fermenting sugars to lure mosquitoes and stimulate them to ingest sugar-based bait laced with a toxic material (e.g., an insecticide). Boric acid is a common ATSB toxicant with demonstrated efficacy against mosquitoes, including *Anopheles* (Müller and Schlein 2008), *Aedes* (Fulcher et al. 2014, Revay et al. 2014) and *Culex* (Müller et al. 2010). ATSB are often placed into "bait stations" to retain the liquid bait and to provide a target for the mosquitoes to land and feed on the bait. The addition of a contact insecticide on bait station surfaces can provide an additional mechanism to kill attracted mosquitoes, with the combination of two control agents (ATSB and contact insecticide) potentially reducing insecticide resistance in wild populations compared to use of a single control agent (Stevenson et al. 2013, Fulcher et al. 2014, Snetselaar et al. 2014).

In the current study, we investigated the use of bait stations containing ATSB, treated in some cases with contact insecticides, for attraction and control of mosquitoes within USDS in the Coachella Valley of southern California. Aqueous ATSB containing boric acid (BA) was stored, dispensed, and protected by bait stations to enhance product longevity, limit the risk of insecticide contamination of sensitive environments, and allow treatment timing and location to be rapidly adjusted. In some trials, additional mosquito control agents composed of an entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Clavicipitaceae) and/or the IGR pyriproxyfen also were applied to bait stations for additional control. *B. bassiana* was selected because experimental applications on USDS chamber walls readily infected and killed *Cx. quinquefasciatus* adults in forced-contact trials (Popko et al. 2018). Pyriproxyfen was tested in USDS because adult mosquitoes contacting a treated surface can transport this material to nearby mosquito development sites that are difficult to treat by conventional means (Fulcher et al. 2014, Snetselaar et al. 2014). The relative efficacy of various bait station designs, ATSB formulations, and treatment combinations including use of fungi and/or pyriproxyfen was explored over a five-year period to determine the viability of ATSB for use in mosquito abatement within USDS.

Methods

Trial Period and Field Sites

Field trials were conducted in two separate regions of the Coachella Valley in southern California from 2018–2022 (Figure 1). Sites within each field trial area were identified in consultation with the Coachella Valley Mosquito and Vector Control District (CVMVCD) based on reported mosquito activity and the presence of underground storm drain systems (USDS) accessible to our research team for application and testing of ATSB designs.

Region #1 (2018–2021) **(Figure 1a-c):** Trials were conducted within the cities of Coachella (33°41′40″ N, 116°11′34″ W), La Quinta (33°43′22″ N, 116°17′20″ W), and Palm Desert (33°46′49″ N, 116°23′12″ W). USDS sites were located within the residential neighborhoods of Coachella and La Quinta and in a gated golf resort complex at Palm Desert. USDS chambers varied in depth, length, and volume across all sites. Standing water > 5 cm in depth was always present at Palm Desert USDS sites, sometimes present in most Coachella sites, and nearly always absent at La Quinta sites.

Region #2 (2022) **(Figure 1d):** Trials were conducted at two adjacent residential communities in the city of Palm Desert (33°45′36″ N, 116°19′06″ W). These residential communities were near a park, elementary school, golf course, and bordered on one side by an interstate highway. All USDS held standing water > 5 cm in depth during the study, although the total amount of standing water varied and was reduced in a few chambers due to sediment accumulation.

Bait Station Design

The progression of bait station designs over the five-year period of field experiments are shown in Figure 2 with bait station components during each trial summarized in Table 1. Bait stations classified as either free-hanging or floating designs were modified over time with the intent to improve mosquito attraction and delivery of mosquito control agents. Free-hanging bait station designs were suspended within USDS chamber structures and used a sponge or water-absorbing beads (Miracle-Gro, The Scotts Company LLC, Marysville OH USA) to draw a liquid sugar solution from a bait reservoir to coat a bait station surface where mosquitoes could access and feed on the sugar bait. Floating bait station designs used plastic food containers as a bait reservoir with sponges to both draw up the liquid sugar solution and to provide a feeding surface for mosquitoes that entered the food container through openings cut into the side of the structure.

Every bait station contained an attractive sugar bait of liquid sucrose (10–33% w:v) and fermented attractant (5–20% v:v) with a small amount of food coloring (1% v:v) and in later trials a mold inhibitor (0.1% w:v) that was mixed the day prior to use in field trials. Boric acid (1% w:v) was added to the sugar bait to make an attractive toxic sugar bait (ATSB). Attractive sugar bait without the boric acid toxicant (ASB-T) was used as a control treatment in several trials. During early trials, the bait contained 10% sucrose, but this was increased to 25–33% sucrose during later years because laboratory studies demonstrated increased mosquito feeding using these higher sucrose concentrations (unpublished data). During each trial, one of three different fermented attractants were used with bait stations to increase mosquito recruitment to and feeding at bait stations. Attractants tested were fermented day-old chick bedding, rodent food, or guava juice. Bait components used and their formulations were selected based on preliminary data of mosquito feeding and

Figure 1.—Field sites. Aerial view of Coachella valley field sites showing underground storm drain systems (USDS) used during the 5-year study period. Field region #1 (2018–2021) included the cities of (a) Coachella, (b) La Quinta, and (c) Palm Desert. Field region #2 (2022) was in (d) Palm Desert, with the dashed line indicating division between the two adjacent communities used in the studies. Specific USDS sites are indicated by map balloons in each image.

subsequent mortality using laboratory-reared *Cx. quinquefasciatus* (unpublished data).

In some trials, additional mosquito control agents were added to the ATSB stations to evaluate mortality when using multi-treatment options. Additional control agents included the entomopathogenic fungus *B. bassiana* (ATSB+BB), the insect growth regulator pyriproxyfen (ATSB+PY), or both agents (ATSB+BB+PY). For ATSB+BB, a commercial formulation of the fungus (BotaniGard® 22WP (BGWP); Laverlam, Butte, MT, USA) was applied as a dry powder at the label-recommended rate to bait station surfaces where mosquitoes might be expected to land or walk across the surface to reach the attractive sugar bait. Spores of this fungus infect adult *Cx. quinquefasciatus* upon contact causing delayed-onset mortality within 1–2 weeks (Popko et al. 2018). In 2021, *B. bassiana* was applied to bait stations as both a dry powder and as a wetted solution using a pressurized hand sprayer allowing application of the fungus to the outer surfaces of the bait station. For ATSB+PY, concentrated pyriproxyfen solution (Pivot™10, Control Solutions Inc., Pasadena TX USA) was diluted as per the label rate for immature mosquito control (to 0.1% v:v) and placed into plastic vials with cotton wicks and also absorbed into water storing crystals. Both vials and crystals containing pyriproxyfen were placed on the top of bait stations where mosquitoes attracted to the bait stations might contact these treatments. For ATSB+BB+PY, dry powder formulations of *B. bassiana* and pyriproxyfen (CAS 95737-68-1, Santa Cruz Technology, Dallas, TX) were homogenized to make a single dry powder formulation (10-25:1 w:w BB:PY) that was applied to all bait station surfaces that mosquitoes might contact to reach the toxic bait. The dry powder formulation of pyriproxyfen was used in this combined treatment because it may be more easily dispersed within the USDS by either mosquito contact or air movement. Dissemination of pyriproxyfen within treated USDS was monitored in 2018–2020 by placing a plastic bowl containing larval food and sentinel larvae (laboratory-reared fourth instar *Cx. quinquefasciatus*) on top of ATSB+PY bait stations ($n = 15$) for 1–2 d, after

Figure 2.—ATSB station designs. Trap designs shown in chronological order (2018–2022). Hanging ATSB designs used in 2018 (a, b, c) and 2022 (k) included an outer support frame and exposed feeding surface with liquid sugar bait. Floating ATSB designs used in 2019–2022 (d–j) included internal bait reservoirs that mosquitoes accessed through an opening in the trap body. Floating designs were later retrofitted with covers (shown in e, g, j) to prevent damage from water in-flows into the USDS chamber.

which larvae were collected and held in the laboratory to evaluate mortality prior to the adult stage.

Bait Station Deployment Strategies

USDS chambers selected for bait deployment trials were easily accessible, with sufficient room inside to set up bait stations and perform mosquito surveillance. Environmental disturbances were common throughout the USDS due to residential activity (e.g., irrigation) and weather events (precipitation, wind) and these impacts constrained how bait systems were designed, deployed, monitored, and processed on-site. Early designs were altered in later trials to protect bait dispensers from catastrophic damage due to wind or water flows, with additional design adjustments focused on increasing bait efficacy. A typical bait station deployment period was 3–4 weeks. During test periods, bait stations were inspected each week, empty bait reservoirs were recharged, and damaged bait stations were removed and excluded from experimental analyses.

Table 1.—Bait station treatments and mosquito surveillance during the 5-year sampling period (2018–2022). Treatments were a bait station with attractive sugar bait (sucrose) and boric acid insecticide (ATSB – attractive toxic sugar bait), bait station containing attractive sugar bait without toxicant (ASB-T), or no bait station (No bait). Additional control agents added to the bait station were *Beauveria bassiana* (+BB) or both *Beauveria bassiana* and *pyriproxyfen* (+BB+PY).

Year	Season	Treatment Distance (m)	Bait Attractant & Nutrient Level [Concentration]	Additional Control Agent(s)	Number of Chambers with Treatment			Number of Aboveground CDC-UV Traps	Number of Chambers Sampled for Larvae	Number of Ovi-tubs in USDS
					ATSB	ASB-T	No Bait			
2018	spring	20	Bedding [20%]	+BB+PY	4	4	4		9	
	fall		Sucrose [10%]	+BB+PY	4[†]	4[†]	4		9	
2019	spring	20	Bedding [20%]	+BB+PY	6	6			8	
	fall		Sucrose [10%]	+BB+PY	6[†]	6[†]		3	5	
2020	fall	200	Rodent Chow [5%]	+BB+PY	6	6		3[‡]	7	
			Sucrose [10%]							
2021	spring	200	Rodent Chow [5%]	+BB	6	6		6[‡]	5	
	fall		Sucrose [10%]	+BB	12[†#]		6[#]	3[#]	7	12
2022	spring*	200	Rodent Chow [5%]	None	10[#] high		6[#]		18	12
			Sucrose [33%]		4[#] low					
	fall		Guava juice [5%]	None	6		6			
			Sucrose [25%]							

[†] Two bait stations were placed in each USDS chamber.
[‡] Aboveground traps were paired with a bait station containing ATSB or ASB-T.
[#] Traps were paired with an ovitub.
* ATSB were deployed in three density/hectare treatments (10 ATSB, 2 ATSB, 0 ATSB) for assessment of area-wide control.

Various bait deployment strategies were implemented during the 11 field trials spanning this 5-year study period (Table 1). A single bait station was normally deployed per USDS chamber, except in three trials when two bait stations per chamber were used. During the first two study years (2018–2019) USDS chambers ∼20 m apart and connected by an underground pipe were selected for paired treatments. One chamber had a bait station with ATSB (Treatment) while the paired chamber had a bait station with ASB-T or had no bait station (Control). During these first trials, it was noted that adult mosquitoes captured in the control chamber occasionally had evidence that they had been exposed to the ATSB in the treatment chamber (bait dye or fungal infection). This suggested movement of adults between nearby USDS chambers. Therefore, in subsequent years, USDS chambers holding different treatments were separated by ∼200 m. Thereafter, mosquitoes from non-ATSB sites rarely exhibited evidence of ATSB exposure indicating that the greater distance effectively limited treatment interactions.

Unique bait strategies explored in a few trials included deployment of stations aboveground, bait stations paired within USDS chambers, or bait stations applied area-wide at low or high density. To increase potential attraction or assess mosquito response to the presence or absence of entomopathogenic fungus, two bait stations were paired within the same USDS chamber during three trials. Paired stations consisted of the same treatments in 2018 and 2019; however, paired treatments differed in 2021, with *B. bassiana* present in one bait station and absent in the other. Sugar baits were differentially colored with food dye to evaluate mosquito feeding, with ATSB (red) and ATSB+BB (green) used to track mosquito feeding at each bait station. In spring 2022, the communities comprising the Region #2 study site were partitioned into three treatment areas to evaluate the effects of bait station density on mosquito abundance within each area. ATSB density per hectare was either 10 ATSB (High), 2 ATSB (Low), or 0 ATSB (Control).

Mosquito Sampling

Adults: Adult mosquito abundance within treatment or control USDS chambers, and occasionally at aboveground locations near USDS chambers, was determined during the weeks before, during and after bait station deployment using a UV-baited CDC-style suction trap (model 512, J. W. Hock Co., Gainesville, FL). A single CDC-UV trap was always deployed in each of 12 USDS chambers (18 chambers in spring 2022) selected as treatment or control chambers. Traps placed within USDS chambers were located at least 0.5 m from the street-side opening to prevent damage from water inflows into the chamber. Mosquitoes captured overnight were transported to the laboratory at UC Riverside where they were placed in a freezer before being separated by sex, reproductive state, and species (Meyer and Durso 1998). The presence of food dye in the abdomen or thorax was recorded as this suggested recent feeding on a sugar bait treatment. Unique to spring 2022, adult abundance was also determined during a three-week intertreatment period between bait station deployment periods.

Immatures: Immature mosquitoes were collected from USDS chambers containing > 5 cm of water depth using a standard 350 mL mosquito dipper cup (Service 1993). Three dip samples were taken in most chambers, one at each end and one in the middle, with a short delay between each dip sample to allow mosquitoes to resume normal activities. Fewer samples were collected when standing water levels were low or solid material had accumulated on one side of the chamber preventing a sample on that side. To standardize across USDS chambers, immature counts for each chamber were the mean of all dip samples taken within the chamber. Dip samples were filtered through a 148 μm mesh screen to retain immature mosquitoes which were preserved in vials with 70% EtOH, sorted by life stage and identified to species (Meyer and Durso 1998). All immature stages (eggs, larvae, and pupae) were counted separately and then counts were combined to generate a total immature count for each sample. Eggs were counted individually rather than as a number of egg rafts because egg rafts were often fragmented as a result of the collection methods.

Oviposition: During two trials in 2021 and 2022, oviposition within USDS chambers was evaluated using a covered plastic washtub (“ovitub”) that contained 1 L of oviposition media (4.3 g hay and 6.26 g nutrient powder (3:1 mouse chow to yeast) per L water fermented at room temperature for one week) (J. W. Hock, 2023). A single ovitub was placed into sampled USDS chambers and in 2021 an ovitub was also placed aboveground near each of the three aboveground adult trapping sites. Each week, two 500 mL subsamples were collected from each ovitub and slowly poured through a filtering screen to collect immature mosquitoes which were otherwise processed as described for immature mosquito sampling above. Any remaining oviposition media was discarded to prevent immatures from completing development to the adult stage. Ovitubs were then rinsed with a small amount of fresh oviposition media before being refilled with fresh oviposition media.

Fungal infection monitoring

During trials using treatments containing the entomopathogenic fungus *B. bassiana* (ATSB+BB or ATSB+BB+PY), mosquito infection with the fungus in each USDS chamber was assessed for up to 100 live or dead adult *Cx. quinquefasciatus* captured by the CDC-UV trap placed in the chamber for adult mosquito surveillance. Selected mosquitoes that were dead within the CDC-UV trap were submerged in 70% EtOH for 20 s to sterilize the mosquito body surface, and then mosquitoes were moved to a fungal observation chamber where they were observed daily for up to 7d for evidence of fungal growth. Selected mosquitoes that were alive in the CDC-UV trap were transferred by aspirator to a 50-dram plastic vial, provided with 10% sucrose solution, and observed daily for mortality for up to 21 d, after which all remaining live mosquitoes were killed by freezing. Mosquitoes found dead within the vial or that were killed at the end of the 21-d period were removed from the vial, surface sterilized, placed in the fungal observation chamber, and observed daily as described above for the dead mosquitoes. Fungal observation chambers were individual wells of a sterile 24-well tissue culture plate, with each well containing a small piece of moist filter paper onto which ≤ 5 dead mosquitoes were placed. Fungal observation chambers were held inside an insect rearing cabinet without light, at room temperature (~20°C) and near 100% humidity. Fungal infection was determined by the combination of fungal growth characteristics (color, structure, size, and shape of hyphae and/or conidia) unique to *B. bassiana* (Stevenson et al. 2013). Infection rates determined were therefore conservative minimum estimates (based on overt signs of infection), since not all fungus-killed individuals produce distinctive fungal growth (Stevenson et al. 2013).

Environmental Conditions

During trials in 2022, temperature and humidity within USDS were monitored hourly using HOBO environmental data loggers (Onset Computer Corp., Bourne, MA USA) suspended from hooks placed on USDS chamber walls at ~1 m below the concrete ceiling of the chamber. A single HOBO probe was placed into each of six arbitrarily selected USDS chambers for the duration of each trial period. Aboveground daily weather data was acquired from a fixed weather station in close proximity to Region #2 (CIMIS: La Quinta II, www.cimis.water.ca.gov).

Statistical Analysis

Analyses were performed using SYSTAT software (version 9, SPSS Inc., 1998). For each trial (separated by year and season), differences among treatments in the number of adult mosquitoes (by sex and reproductive status) or immature mosquitoes (all immature life stages combined) collected within USDS were determined using non-parametric rank tests (Mann-Whitney U-test for trials with two treatments or Kruskal-Wallis for trials with three treatments). Adult mosquito capture in aboveground traps was similarly evaluated against adult mosquito capture within USDS using non-parametric rank tests. Treatment differences were considered significant at $\alpha = 0.05$.

Results and Discussion

Environmental Conditions

Mean air temperature recorded by the aboveground weather station near the Region #2 study site in 2022 was 27°C in spring and 17°C in fall, ranging from a low of 3°C in fall to a high of 51°C in spring (Figure 3). Temperature ranged more narrowly within the USDS, with a mean temperature of 26°C in spring and 22°C in fall, and with a low of 10°C in fall and a high of 41°C in spring. Relative humidity aboveground was 31% in spring and 39% in fall, ranging from a low of 7% in spring to a high of 79% in fall.

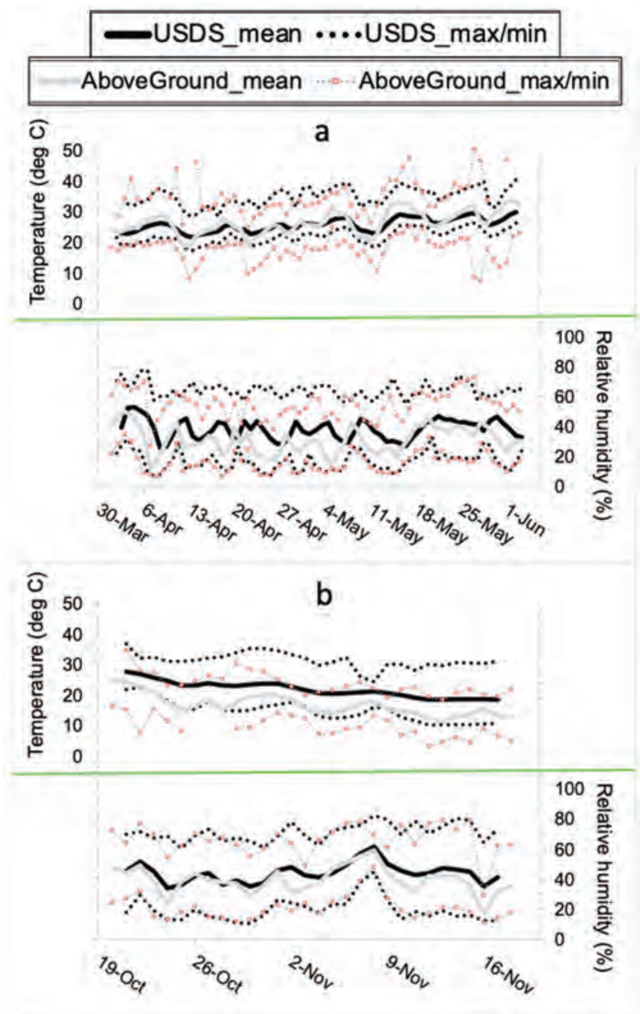

Figure 3.—Environmental conditions during 2022 trials. Air temperature (top) and relative humidity (bottom) within USDS chambers (Region #2) compared to aboveground conditions recorded by a nearby weather station. Daily mean, minimum, and maximum are displayed for spring (a) and fall (b) trials.

Within the USDS, relative humidity was typically higher than that recorded aboveground, with a mean relative humidity of 38% in spring and 44% in fall, and ranging from a low of 8% in spring to a high of 82% in fall. In the spring, very low relative humidity (< 10%) was commonly recorded both aboveground and within the USDS. Relative humidity during fall always exceeded 10%.

Mosquito abundance

Adults: Over the five-year study period, 85,465 adult mosquitoes were collected in CDC-UV traps placed within USDS chambers, with an overall mean(CI) of 95(85–105) adult mosquitoes per trap-night ($n = 898$ trap-nights). Traps with > 500 mosquitoes in a single night were not uncommon ($n = 27$) with a maximum single night trap capture of 1,807 mosquitoes during spring 2021. Aboveground CDC-UV traps captured

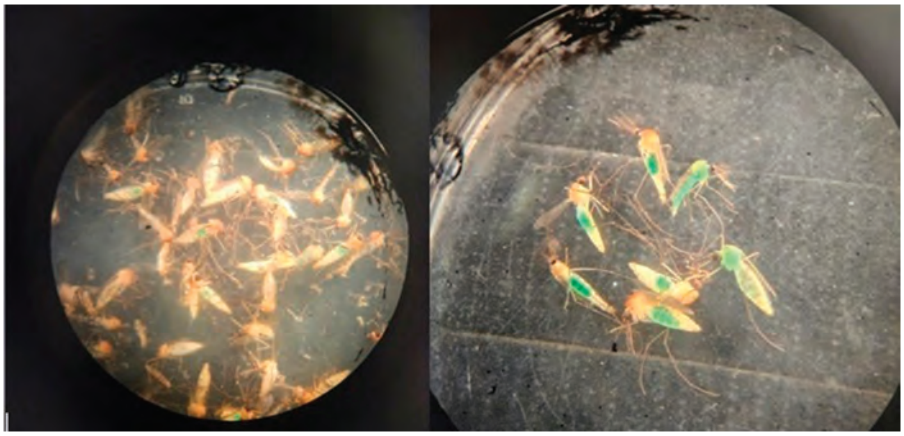

Figure 4.—*Culex quinquefasciatus* adults with green coloring indicative of bait ingestion in an unsorted sample (left) and in isolated bait-positives (right) from CDC-UV traps.

substantially fewer mosquitoes relative to traps placed in USDS chambers (U = 260–5115; $p \leq 0.02$), with an overall mean(CI) of 2(1.2–2.9) adult mosquitoes per trap-night ($n = 93$ trap-nights) (Figure 5).

Bait-feeding was evident (Figure 4) in < 1% of total adults (n = 583) collected from CDC-UV traps, with no more than 2% of mosquitoes being bait-fed during any trial regardless of bait station design or bait formulation. Nearly all (99%) bait-colored mosquitoes were captured within USDS chambers containing a bait station, with the remainder captured within nearby chambers lacking a bait station. No bait-fed mosquitoes were captured in traps deployed aboveground. Few bait-fed mosquitoes were detected in all trials, regardless of station design, distribution and density, and similar proportions of bait-fed mosquitoes were captured in USDS chambers with either the ATSB or ASB-T treatments deployed concurrently.

Nearly all (> 99%) of the adult mosquitoes collected were *Cx. quinquefasciatus,* with a 2:1 (F:M) sex ratio. Females were 63% non-gravid, 28% gravid, 5% bloodfed, and 3% undetermined. Other species captured were *Cx. tarsalis* Coquillett (0.5%), *Aedes aegypti* (L.) (0.06%), *Culiseta inornata* (Williston) (0.03%), *Cs. incidens* (Thomson) (< 0.01%) and *Cx. stigmatosoma* Dyar (< 0.01%). Adult *Ae. aegypti* were first collected from USDS chambers in spring of 2018 and continued to be collected from these sites in low numbers during subsequent years.

Although adult mosquito abundance appeared to differ by treatment during one or more weeks in some trials, these differences were often inconsistent across a single trial period as well as among trials in different years and seasons (Figure 5, Table 2). During two trials with a significant treatment effect (spring 2018 and spring 2022), more female mosquitoes were captured in USDS chambers with the ATSB treatment than in USDS chambers with the control treatments (ASB-T and/or No Bait). Additionally, in spring 2022 more female mosquitoes were captured in USDS chambers with ATSB in the treatment area with a low density of bait stations relative to the high-density treatment area. When considered across all trials, treatment (ATSB, ASB-T, No Bait) appeared to have had no effect on adult female mosquito abundance within the USDS chambers. Furthermore, the relative number of adult mosquitoes captured within USDS chambers treated with ATSB during each trial showed little change (and was rarely reduced) during bait station deployment periods relative to pre- or post-deployment periods (Figure 5).

Immatures: Over the five-year study period, a total of 106,813 immature mosquitoes were collected by dipper with an overall mean(CI) of 68(59–77) mosquitoes per dip sample (N = 1,572 dip samples). Of the immature mosquitoes collected, 48% were young larvae ($1^{st}/2^{nd}$ instars), 31% mature larvae ($3^{rd}/4^{th}$ instars), 17% unhatched eggs, and 4% pupae. Nearly all (> 99%) of the mature larvae were *Cx. quinquefasciatus* with the few other species identified as *Cx. tarsalis* ($n = 51$), *Cx. stigmatosoma* ($n = 5$), and *Ae. aegypti* ($n = 1$). The single *Ae. aegypti* larva was collected in Palm Desert during fall of 2021.

The number of immature mosquitoes collected by dip sample within USDS chambers varied inconsistently over time both within and between treatments, although the number of immature mosquitoes was often higher in USDS chambers with the ATSB treatment relative to the control treatment (ASB-T or No Bait) (Figure 6). Additionally, the relative number of immature mosquitoes did not change substantially in any treatment group during bait deployment periods relative to pre- or post-deployment periods.

Oviposition Activity: A total of 689,656 immature mosquitoes were recovered from 352 ovitub subsamples during 2021 and 2022. Immatures were 54% young larvae ($1^{st}/2^{nd}$ instars), 34% unhatched eggs, 11% mature larvae ($3^{rd}/4^{th}$ instars) and < 1% pupae. All mature larvae were *Cx. quinquefasciatus*, except for a single *Cx. tarsalis* in spring 2022 and two *Ae. aegypti* (3^{rd} and 4^{th} instars) in fall 2021.

Immature abundance increased over the treatment period in each trial, with immature abundance in ovitubs at the end of the trial period being 100-fold greater in fall 2021 and 23-fold

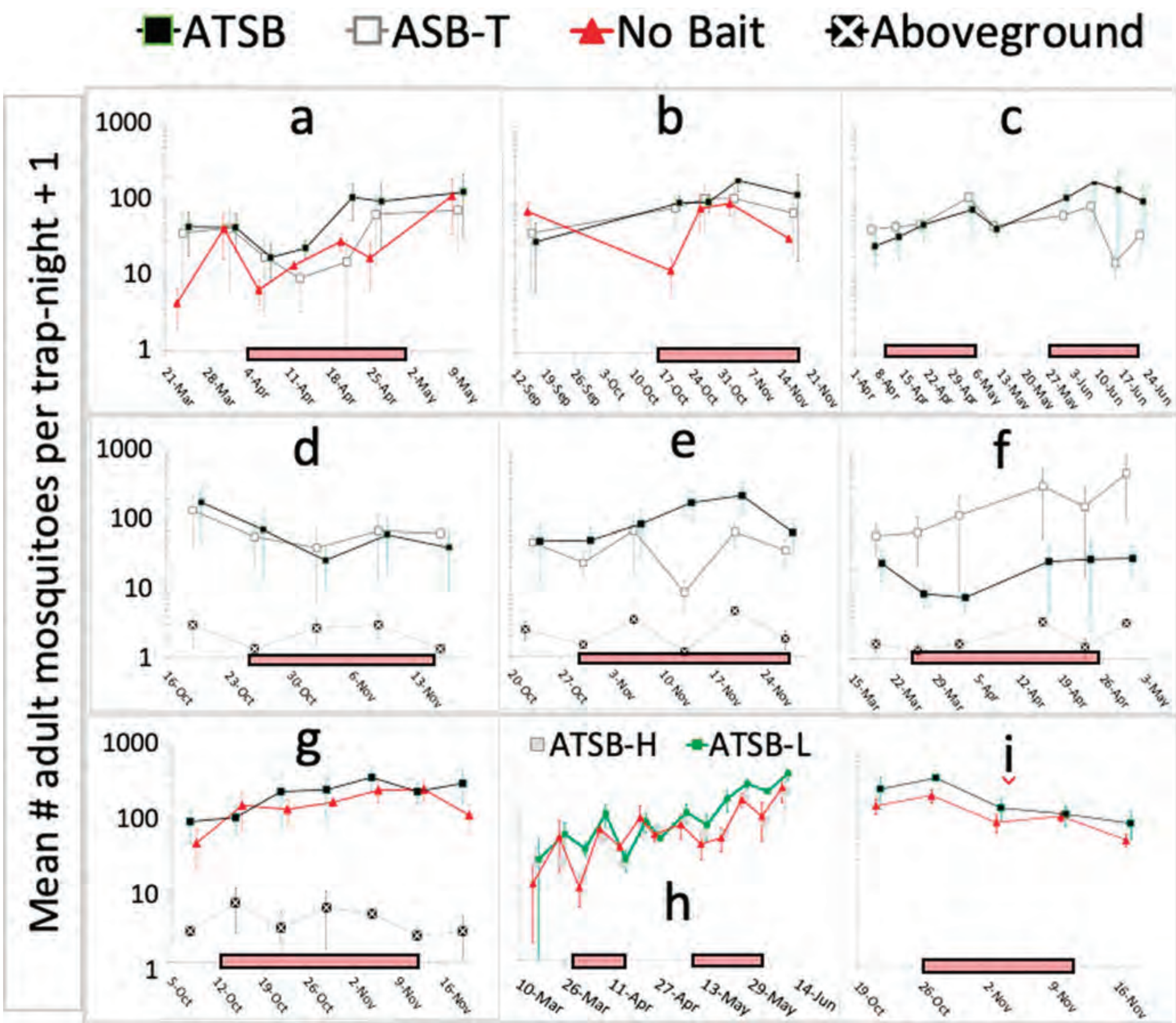

Figure 5.—Adult mosquito abundance (mean ± SE on a $\log_{10}$ scale) in CDC-UV traps by season and year (a) spring 2018, (b) fall 2018, (c) spring 2019, (d) fall 2019, (e) fall 2020, (f) spring 2021, (g) fall 2021, (h) spring 2022, and (i) fall 2022. Traps were placed within USDS chambers containing bait stations with attractive toxic sugar bait with boric acid toxicant (ATSB) for comparison to mosquito abundance in USDS chambers containing bait stations with the sugar bait but without the toxicant (ASB-T) and/or USDS chambers without a bait station (No Bait). Occasionally, traps were also placed aboveground near USDS chambers (AboveGround). During fall 2022, bait stations were placed into USDS chambers within different regions of the study site at one of three ATSB densities: high density (10 ATSB/hectare) (ATSB-H), low density (2 ATSB/hectare) (ATSB-L), or no bait stations (No Bait). Shaded bar along x-axis (date) indicates periods that bait stations were deployed within USDS chambers during each seasonal trial.

greater in spring 2022 (Figure 7). There was no effect of treatment on immature abundance in ovitubs placed within USDS chambers in spring ($H = 1.551$, $P = 0.461$) or fall ($U = 639.0$, $\chi^2 = 0.280$, $P = 0.597$). However, ovitubs placed in USDS chambers had a significantly greater number of total immatures than those placed aboveground ($U = 394.5$, $\chi^2 = 3.144$, $P = 0.076$), largely due to a greater number of eggs and mature larvae in USDS chamber ovitubs ($U < 362$, $\chi^2 > 4.802$, $P < 0.03$), with numbers of young larvae being similar in ovitubs within USDS chambers and aboveground ($U = 441.0$, $\chi^2 = 1.566$, $P = 0.211$).

Fungal infection and pyriproxyfen dissemination

When *B. bassiana* was present in bait stations (Table 1), sporulation from dead *Cx. quinquefasciatus* was observed in 145/3,852 adult females (3.8%) and 26/1,171 adult males (2.2%) sampled from CDC light traps. All fungus-positive adult mosquitoes were collected when dry powder was applied to stations and no fungus was observed when fungus was applied as a wet spray. Overall, 62% of infectious individuals were collected within USDS chambers containing bait stations treated with fungus (ATSB+BB or ATSB+BB+PY). The

Table 2.—Non-parametric analysis of adult mosquito abundance from CDC-UV traps in USDS among treatments by sample year and season. Only dates during bait deployment were included in the analysis. [CI] = 95% confidence interval with upper and lower values. (U) = Mann-Whitney tests in the row; (H) = Kruskal-Wallis ANOVA in the row. Low = Low density bait station treatment. High = High density bait station treatment.

		Total Adult Female Mosquitoes					Non-gravid		Gravid		Bloodfed		Males	
		Mean number per trap-night [CI]												
Year	Season	ATSB	ASB-T	No bait	Statistic	*P*-value	Statistic	*P*-value	Statistic	*P*-value	Statistic	*P*-value	Statistic	*P*-value
2018	Spring	35.3 [19/51]	22.5 [7/38]	13.6 [7/20]	(H) **8.436**	**0.015**	5.679	0.058	4.362	0.113	0.905	0.636	1.895	0.388
	Fall	130 [68/194]	99.3 [41/151]	48.4 [20/76]	(H) 5.569	0.062	4.767	0.092	5.652	0.059	**6.495**	**0.039**	0.782	0.676
2019	Spring	70.7 [35/106]	45.6 [29/63]		(U) 1405	0.871	1506	0.642	1268	0.309	1421	0.946	1411	0.901
	Fall	43.4 [21/66]	45.0 [15/75]		(U) 408.5	0.539	395.5	0.420	436.0	0.836	482.5	0.566	464.0	0.833
2020	Fall	80.4 [39/122]	25.4 [10/40]		(U) 458.0	0.070	467.0	0.087	464.0	0.080	505.0	0.188	**440.0**	**0.042**
2021	Spring	7.88 [3/13]	68.7 [25/112]		(U) **952.0**	**0.001**	**982.5**	**<0.001**	**869.5**	**0.003**	**864.5**	**0.014**	**845.0**	**0.025**
	Fall	182 [112/252]		132 [85/180]	(U) 998.0	0.299	1035	0.171	932.5	0.651	895.0	0.907	990.5	0.331
2022	Spring	High: 49.3 [34/65] Low: 82.6 [63/102]		44.6 [28/61]	(H) **4646**	**0.034**	4779	0.066	**4140**	**0.001**	5082	0.209	4942	0.137
	Fall	130 [73/187]		107 [41/174]	(U) 334.0	0.086	570.0	0.076	533.0	0.219	502.5	0.419	**229.0**	**0.001**

remaining 38% of fungus-positive adults were discovered from a single USDS chamber without fungus that was connected by an open underground pipe to a chamber across the street that had a bait station containing the fungus. It seems that either few mosquitoes contacted the fungus when applied to a bait station or mosquitoes were not readily infected with the fungus under the field conditions experienced in this study. No mosquitoes with a fungal infection were captured in CDC traps before bait stations with the fungus were deployed.

There was little evidence of PY dissemination to sentinel larvae placed above bait stations containing ATSB+BB+PY (during 2019-2020 trials). In fall of 2020, mortality of sentinel larvae averaged less than 10% in all stations and was similar in chambers with or without the PY treatment, both within USDS and at aboveground sites, except that high mortality (85%) occurred in sentinel larvae at three above ground sites on a single date (April 7) when high winds occurred.

Discussion

When considered over all trials, bait stations containing attractive toxic sugar baits (ATSB) placed within underground storm drain systems (USDS) were ineffective to reduce numbers of adult mosquitoes within these USDS. Although many factors could have rendered ATSB applications ineffective, the design of bait stations was a key hurdle. Over the 5-year study, initial bait station designs were unsuited for the USDS environment and were often damaged by water inflows. Later designs added bait station covers and enclosed containers to protect against street-level disturbances, but these measures likely limited mosquito contact with the ATSB held within bait stations. Laboratory studies (unpublished) showed reduced efficacy of bait stations against *Cx. quinquefasciatus* when covers were added to bait stations. Although later bait station designs provided both protection and improved mosquito access when tested in the laboratory, under field conditions the proportion of mosquitoes within the USDS chamber that fed on the bait remained low. Bait stations with greater exposure of ATSB to mosquitoes were also more prone to drying and hardening of the sugar bait over the deployment period. Preliminary laboratory studies indicated reduced mosquito feeding on dried sugar baits and drying of the sugar bait under field conditions likely reduced feeding by adult mosquitoes. Bait stations with larger bait reservoirs and with drying-resistant feeding interfaces would be critical to prolong ATSB activity in the field. Although the final bait station design (Fig. 2k) had an easy-to-refill reservoir, larger feeding surface, and weekly bait replacement ensuring bait station viability for at least one month, even this design with ATSB failed to reduce mosquito numbers within treated USDS chambers.

Over the 5-yr project period, adjustments were also made to the ATSB formulation with the intent of improving mosquito feeding on the bait and ultimately to improve efficacy of bait stations in reducing mosquito numbers

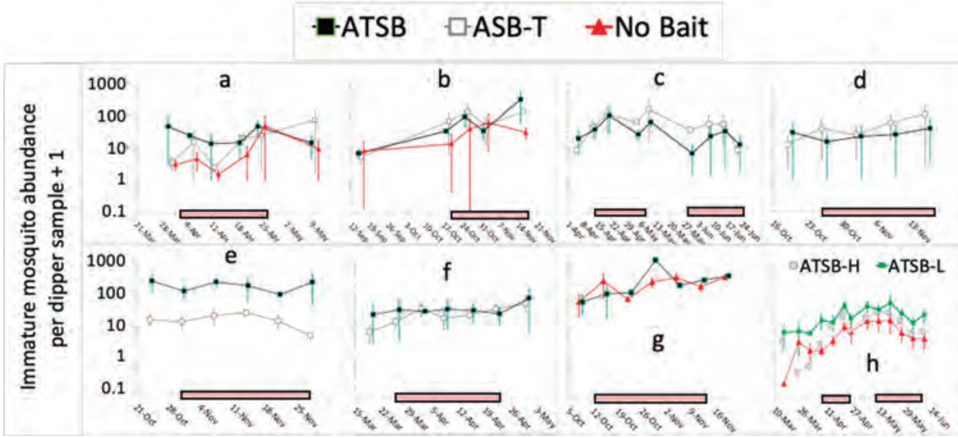

Figure 6.—Immature mosquito abundance. Number of immature mosquitoes (mean ± SE on a $\log_{10}$ scale) in dip samples from USDS chambers containing standing water by season and year: (a) spring 2018, (b) fall 2018, (c) spring 2019, (d) fall 2019, (e) fall 2020, (f) spring 2021, (g) fall 2021 and (h) spring 2022. Treatments tested in USDS chambers were ATSB (attractive toxic sugar bait with boric acid toxicant), ASB-T (attractive sugar bait without toxicant), or No Bait (chamber lacked a bait station). Shaded bar along x-axis (date) indicates the period that bait stations were deployed within USDS chambers during each seasonal trial.

within treated USDS chambers. Initial ATSB formulations utilized 10% sucrose and 10% fermented chicken bedding to provide the attractive volatiles to encourage mosquito orientation to the bait station and to encourage mosquito feeding on the bait. Based on later laboratory assays (unpublished data) the attractant was changed to 5% fermented mouse chow and then later to 5% fermented guava juice. The sucrose concentration was also adjusted following laboratory studies that showed increased mosquito feeding on ATSB with sucrose concentrations $>$ 10% (up to 75% sucrose) leading to a final ATSB formulation with 25% sucrose to increase mosquito feeding while being more resistant to drying formulations with higher sucrose concentrations.

Mosquito feeding on the sucrose bait also may have been impacted by use of the food-grade preservative, potassium sorbate, which was added to baits in later studies to inhibit mold growth. In initial no-choice assays conducted in the laboratory (unpublished data), addition of this preservative did not reduce feeding and thus it was used with bait in field studies. However, later choice assays conducted in the laboratory suggested potassium sorbate may reduce adult feeding on baits when other food sources were available to mosquitoes (unpublished data). Over several years of field studies, mold growth in ATSB at field sites was of limited concern, possibly due to high ventilation of field settings. The addition of a preservative to ATSB is therefore not recommended.

Limited mosquito feeding on ATSB, perhaps due to the factors described above, is likely most responsible for the lack of treatment effect when considering all trials. However, use of 1% boric acid (BA) as the toxicant also must be considered. Although other ATSB studies have similarly used 1% BA and laboratory assays using 1–5% BA as a toxicant with ATSB demonstrated ∼90% mortality at the 1% BA concentration (unpublished data),

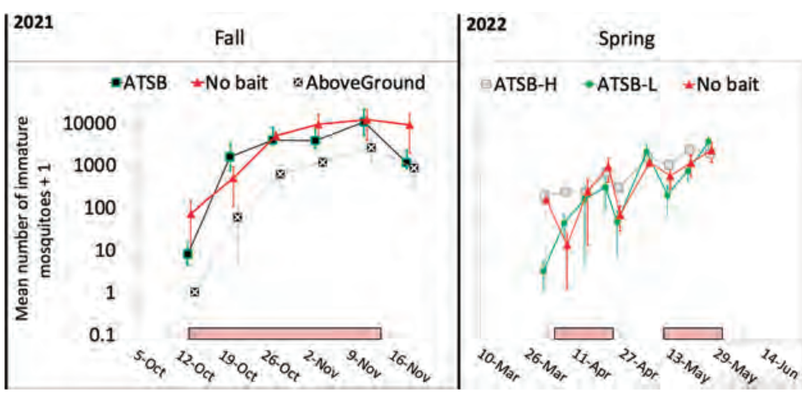

Figure 7.—Oviposition activity. Abundance (mean ± SE on a $\log_{10}$ scale) of all immature life stages of *Culex* spp. in subsamples taken from plastic tubs with oviposition attractive infusions (“ovitubs”) placed within USDS chambers. Abundance shown by bait treatment for each trial. Shaded bar along x-axis (date) indicates the period that bait stations were deployed within USDS chambers during each seasonal trial.

mosquito mortality is relatively slow with mortality typically occurring 3–7 days after exposure. The slow mortality rate following exposure to BA may have resulted in a limited reduction in the number of adult mosquitoes within treated USDS chambers, and perhaps even allowed gravid females to oviposit prior to death. A faster acting toxicant should be considered for future studies using ATSB.

The inclusion of additional control agents (*B. bassiana* (BB) and pyriproxyfen (PY)) to bait stations also failed to reduce adult mosquito abundance within USDS chambers relative to either ATSB with boric acid alone or even no bait controls. BB infections detected in adult mosquitoes from light traps confirmed mosquitoes could be infected with BB acquired from a bait station, although low transmission rates indicated that BB transmission was inefficient in these trials. Laboratory findings indicated that a dry BB formulation (but not a wet spray formulation) can reduce *Cx. quinquefasciatus* feeding on sugar bait, thereby inhibiting overall performance of ATSB when BB is also applied (unpublished). Although BB applied in a spray to USDS chamber walls can infect and kill significant proportions of adult *Cx. quinquefasciatus* forced to contact treated surfaces (Popko et al. 2018), BB spray treatments applied to bait stations in the current study did not seem to be sufficiently infectious, perhaps due to limited mosquito contact with the BB treatment. There was also little evidence for dissemination of pyriproxyfen from bait stations when PY was applied either as a wet solution or as a dry powder. Sentinel larvae placed above bait stations with PY had low mortality that was not different from control treatments, suggesting PY was not disseminated within the USDS chamber by adult mosquitoes or by wind. Based on these results, neither BB nor PY enhanced bait station efficacy and their inclusion is not recommended for use with bait stations placed within USDS chambers in the Coachella valley.

Acknowledgements

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Field evaluation of long-acting residual mosquito control product, Altosid® XR Briquets, in neglected swimming pools in Orange County, CA

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Introduction

Leaving a swimming pool stagnant for an extended period can lead to various issues, including the proliferation of algae, bacteria, and other microorganisms. A neglected pool can also accumulate abundant organic matter, leaves, and other debris, creating a suitable habitat for mosquito larval development. If left untreated, a single neglected pool can potentially be the source of thousands of adult mosquitoes, resulting in nuisance biting and an increased risk of mosquito-borne pathogens and their transmission (Reisen et al., 2008). Neglected swimming pools may be discovered within privately owned backyards, increasing the difficulty of locating and treating these important mosquito sources. With a population of approximately 3.2 million people (US (United States) Census Bureau, 2020), Orange County, California, is home to more than 110,000 backyard swimming pools (CAPE Analytics [WWW Document], 2020).

In 2023, Orange County Mosquito and Vector Control District (OCMVCD or "District") staff detected 3,364 neglected pools, a 26% increase from 2022. Among these neglected pools, field technicians categorized 2,369 (~70%) as "active" or "rain" pools, and these pools required 2–4 inspections each during the season. Field technicians categorized the remaining 995 pools (~30%) as "archived," which means a homeowner took actions to decommission the pool completely (e.g., filling it in with dirt), or restored the pool to operational condition. In 2023, OCMVCD conducted 8,265 inspections, totaling 1,593 inspection hours at a cost of more than $138,000.

To reduce the number of repeated visits to neglected backyard swimming pools, field technicians primarily treat neglected pools with two residual-acting products containing either spinosad (IRAC 5) or the insect growth regulator (IGR) S-methoprene (IRAC 7A) to extend retreatment cycles. However, despite regular product rotations to avoid insecticide resistance, technicians have voiced concerns regarding the long-term efficacy of Altosid® XR briquets (Zoecon, Schaumburg, IL). According to its label, Altosid XR briquets contain 2.1% S-methoprene and are effective at reducing adult mosquito production in water sources for up to 150 days post-treatment when applied according to label instructions.

For effective mosquito control, it is important that control strategies achieve close to 100% efficacy. The World Health Organization (WHO) sets the threshold for effective mosquito control using larvicides, including IGRs, at ≥ 90% (WHO, 2005). A recent study (Evans et al., 2023) demonstrated that individual mosquito larval survival and adult emergence increased when control strategies only killed a portion of the larvae, resulting in greater mosquito production from inadequately treated sources. From 2015-2020, OCMVCD conducted semi-field pesticide efficacy tests with several Altosid formulations (pellets, 30- and 150-day briquets) with *Culex quinquefasciatus* Say larvae in containers ranging in volumes from 18.9-1900 L (5-500 gal). Results showed that adult emergence exceeded 30% for all formulations in sources > 380 L (100 gals) (unpublished data). In 2023, we evaluated the performance of Altosid XR briquets in neglected swimming pools in response to concerns raised by District staff.

Methods

We selected 19 neglected swimming pools with larvae in areas of Orange County and treated each pool with the maximum number of Altosid XR briquets by volume following label instructions. *Culex quinquefasciatus* was the species collected most often in neglected swimming pools in Orange County (Nguyen et al., 2010). After several weeks post-treatment, field technicians returned to the treated pools and collected a water sample (~ 5.7 L, 1.5 gal) in designated 19 L (5 gal) buckets from each pool. Mosquito larvae and pupae were collected using a fine mesh pool skimmer and released into individually labeled buckets. The elapsed time between the treatment date and the date on which the technician collected the water and immature mosquito samples ranged from 23-104 days.

In the District laboratory, a small water sample (~250 ml) from each bucket was transferred to an individually labeled plastic emergence chamber (Bioquip, Rancho Dominguez, CA). We then added 20-50 pupae from each bucket to the

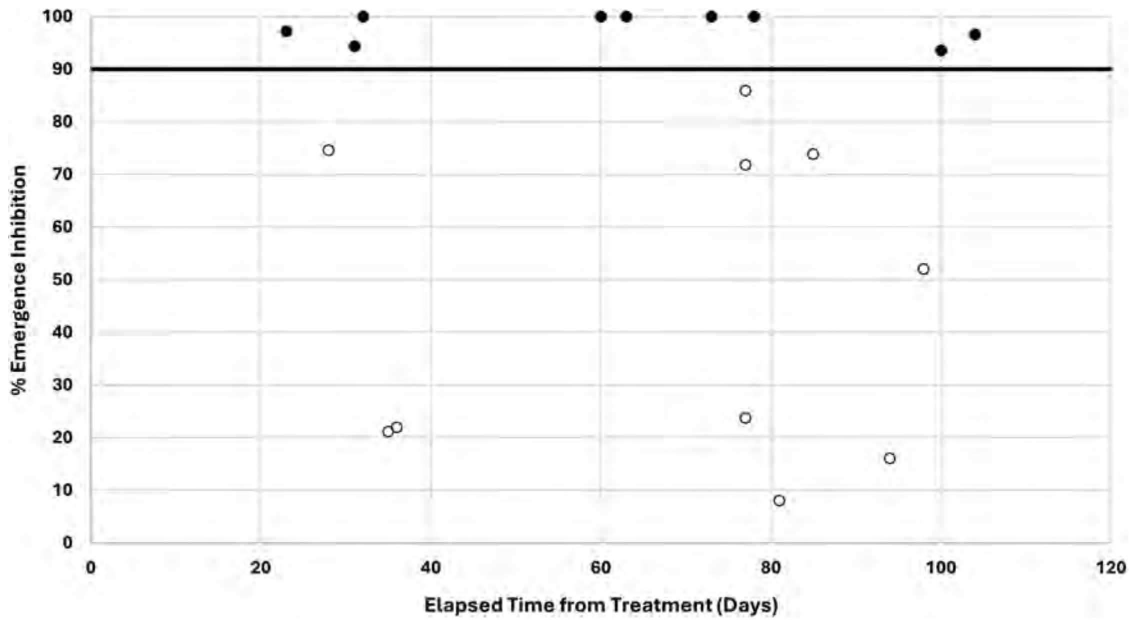

Figure 1.—Emergence inhibition (EI) of adult mosquitoes in neglected swimming pools treated with Altosid XR Briquets in Orange County, CA plotted as function of time elapsed after treatment. Closed circles above the 90% emergence inhibition threshold represent swimming pools with acceptable control and the open circles below the line indicate treatment failures.

corresponding water sample in the respective emergence chamber. If there were not enough pupae collected from a treated swimming pool, we substituted 3rd and 4th instar mosquito larvae from the bucket in lieu of pupae. We set aside two emergence chambers as untreated controls, filled them with 250 ml of tap water and added 50 *Cx. quinquefasciatus* pupae collected from a mosquito rearing basin on District property to both control chambers. The 19 emergence chambers and two untreated controls were maintained on an 18:6 L:D cycle at 25.5° C in the insectary for one-week and the percent emergence inhibition recorded (% EI, WHO, 2005) on the 7th day. We used the Henderson-Tilton formula (Henderson and Tilton, 1955) to correct the percent emergence inhibition for the natural mortality observed in the control emergence chambers. Emerged adults were enumerated and identified to species and sex using taxonomic keys (Meyer and Durso 1998). Only emerged *Cx. quinquefasciatus* adults were used to evaluate product efficacy in this study.

Results and Discussion

Our field assessment revealed inconsistent efficacy of Altosid XR briquets, with an average emergence inhibition of 70.2% ($\pm$ 17.6%) for late-stage *Cx. quinquefasciatus* larvae and pupae. Ten (53%) of the 19 neglected pool samples exhibited adult emergence rates $>$ 10%, exceeding the minimum effective control threshold of 90% EI (Figure 1). No relationship was found between emergence inhibition and days elapsed after treatment. These results align with previous field (Phillips et al., 1991) and semi-field studies (Sus, 2023), where average emergence inhibition $<$ 70% were recorded for *Cx. quinquefasciatus* pupae collected from catch basins treated with Altosid XR briquets. The reasons for the failure of Altosid XR briquets to adequately control *Cx. quinquefasciatus* may be due to the development of low-level resistance to S-methoprene after decades of applications (Su and Cheung, 2014), insufficient release of the IGR from the briquette, or a combination of these two factors.

Conclusion

Altosid XR Briquets showed limited efficacy in controlling mosquito larvae in neglected swimming pools with substantial water volumes. Based upon these results and earlier semi-field studies, the District has discontinued the use of Altosid XR briquets in neglected swimming pools with water volumes $>$ 380 L (100 gal). To assist field technicians, OCMVCD created a pesticide recommendation chart to provide alternative products to replace Altosid XR briquets in large peridomestic water sources. Moving forward, the District would like to expand the use of mosquitofish (*Gambusia affinis*) to control mosquito larvae in neglected pools capable of sustaining mosquitofish year-round.

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Visualizing risk factors for local arboviral disease transmission by the *Aedes aegypti* mosquito within the service area of the Greater Los Angeles County Vector Control District

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Abstract

To better prioritize our response to imported cases of arboviral disease transmissible by the *Aedes aegypti* mosquito, we developed a risk assessment model that can be easily visualized. Here we discuss the factors included in our risk determination and the parameters used to create a risk assessment map.

Evaluation of Wide Area Larvicide Spraying (WALS) efforts in Kern County, California

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Abstract

Aedes aegypti (L.) (Diptera: Culicidae) is a day biting mosquito capable of transmitting a variety of arboviruses. Truck mounted Wide Area Larvicide Spraying (WALS) applications were conducted in Bakersfield, CA, USA by the Kern Mosquito and Vector Control District during the summers of 2022 and 2023 to investigate the effectiveness of WALS in reducing the *Ae. aegypti* population in treatment areas. Five weekly traps were placed in three treatment zones and the number of *Ae. aegypti* collected were compared with a control zone. Larval bioassays determined the residual efficacy of WALS in treated containers and the effects of cover and distance on spray distribution. During the 2022 WALS applications, a significant reduction in the average trap count was found in treatment zones compared with the control. However, we did not find a significant reduction following the 2023 WALS applications. There was no significant difference in percent larval mortality in cups placed in front or back yards of residences. Similarly, we did not find a significant reduction in larval mortality that were unobstructed compared with those that were placed under cover such as vegetation. Our results demonstrate that while WALS applications can be successfully utilized to manage *Ae. aegypti* populations, aspects such as the timing of applications are important for effective use.

Introduction

Aedes aegypti (L.) (Diptera: Culicidae) is a vector of global importance, capable of transmitting arboviruses such as dengue, Zika, chikungunya, and yellow fever (Lwande et al. 2020). These viruses constitute a major disease burden globally, with a yearly estimated 390 million cases of dengue worldwide (Bhatt 2013). This day biting mosquito often exploits cryptic larval sources, making traditional control difficult (Cheong 1967, McGregor and Connelly 2021). Area wide treatment techniques such as Wide Area Larvicide Spraying (WALS) can potentially enable the effective treatment of these hard to treat larval habitats (Sun et al. 2014, Williams et al. 2022). WALS applications typically utilize the microbial active ingredient *Bacillus thuringiensis israelensis* Barjac as a larvicide. The ability of WALS to target cryptic sources makes this an attractive method to incorporate into existing mosquito management strategies.

Aedes aegypti was first detected in California in 2013 and is currently present in 24 counties throughout the state (Gloria-Soria 2014, CDPH 2024). *Aedes aegypti* first reached Kern County in 2014 and has since become firmly established. The objectives of the current study were to determine the effectiveness of WALS applications in reducing the population of *Ae. aegypti*. To do this, a series of truck mounted WALS applications were conducted in Bakersfield, CA over two consecutive summers within several treatment zones. Adult surveillance was conducted within these zones to evaluate the effectiveness of WALS treatments for *Ae. aegypti* control. Larval bioassays were conducted to evaluate the effects of distance, cover and residual efficacy of WALS applications by assessing 24 hour larval mortality.

Materials and Methods

WALS Applications

WALS applications were carried out by the Kern Mosquito and Vector Control District (District) during June - July of 2022 and 2023 in Bakersfield, CA, USA. Each summer, three treatment zones (1 mi^2 in 2022 and $\frac{1}{2}$ mi^2 in 2023) were chosen based on previous surveillance data indicating that large populations of *Ae. aegypti* were present in these areas. A comparable control zone which received no WALS treatments was also selected. Zones were spread across Bakersfield and did not border any other zone. VectoBac WDG (AI: *Bacillus thuringiensis israelensis*; Valent Biosciences, Libertyville, IL, USA) was applied at 4 oz/acre using a truck mounted A1 Super Duty system during the late night/early morning. Each zone was treated weekly for four consecutive weeks, followed by two biweekly treatments. In 2023, the first scheduled WALS application was not conducted due to adverse weather and an additional two treatment zones were not treated due to mechanical failures, one each during the second and third applications. Normal mosquito control efforts (e.g., source reduction, pool treatments, service requests) by the District continued uninterrupted in both the treatment and control zones for the duration of the surveillance period.

Adult Mosquito Surveillance

To monitor for adult mosquitoes, five BioGents Sentinel (BGS) traps (BioGents AG, Regensburg, Germany) were set in each of three treatment and one control zones weekly for 24 weeks in 2022 and 29 weeks in 2023. Only one trap per zone was set during the final week of 2023. Traps were baited with CO_2 (dry ice) with no scent lure. Traps were set in the front yard of residences in the late morning/early afternoon and collected the following morning. Mosquitoes were returned to the District laboratory, anesthetized with triethylamine, counted and identified to species. Trapping efforts began four (2022) or eight (2023) weeks prior to the first WALS applications, continued throughout the treatment period and ended 12 (2022) or 14 (2023) weeks after the final application.

Larval Mortality

To determine the effects of distance and cover on WALS effectiveness, four empty paper cups (16 oz, EcoQuality, Brooklyn, NY, USA) with a small stone for weight were placed at three residences the day prior to treatment and collected the following day. Two were placed in the front yard and two were placed in the back yard. One in each location was placed with no vertical obstructions, while one was placed such that there was an obstruction (e.g., vegetation) directly above the cup. Untreated cups were used as the control. Cups were collected and 200 mL of water was added to each cup, along with a small amount of ground cat food. Twenty field collected mosquito larvae (2^{nd}, 3^{rd}, or 4^{th} instar) were added to each cup and mortality was checked after 24 hours. Field mosquito larvae were collected weekly by district operations staff for use in mortality studies. Due to our requirements for a large number of mosquitoes, both *Culex* spp. and *Ae. aegypti* were used.

To determine the duration of WALS efficacy within a container, a similar study was conducted in 2023. Four empty cups containing a rock were placed in the front yards of three residences in each treatment zone. These were set the day before treatments and collected the following morning. To determine the effectiveness of WALS applications on dry containers which later were filled with water (e.g., via irrigation or rain), these four cups were divided into two groups, those that would have water (200 mL) added upon collection (immediate) and those that remained dry for two weeks (delayed) prior to water being added. Twenty field collected larvae (*Culex* spp. or *Ae. aegypti*) were added to each cup and the percent mortality was determined the following day. Larval mortality was monitored in each cup weekly for ten weeks following collection. This experiment was repeated following four WALS applications, so each of the two groups (immediate/delayed) had 24 replications in each of the three treatment zones. Untreated cups were used as the control.

Statistics

Negative binomial regression was used to compare BGS trap counts of adult (males + females) *Ae. aegypti* in the treatment and control zones. Trap counts from the three treatment zones were combined into one group for analysis. Traps that did not contain any *Ae. aegypti* were included in the analysis as a zero. Rosner's outlier test was used to confirm suspected outliers among the adult mosquito collections and larval mortality studies (Rosner 1975). A two-way unbalanced ANOVA was used to compare the effects of both distance and cover on larval mortality following a WALS application. Wilcoxon rank sum test and Kruskal-Wallis test with Dunn's multiple comparisons were used to determine the effect of time on larval mortality following WALS applications. All statistical analysis was done using R version 4.2.1 (R Core Team 2022).

Results and Discussion

During the 2022, there were 336 BGS traps placed in the treatment zones and 120 set in the control zone. Of these, eight (five treatment, three control) were removed from the analysis due to various issues (e.g., trap failures). Three species (*Ae. aegypti*, *Culex tarsalis*, *Cx. quinquefasciatus*) comprised $\approx 99.6\%$ of the total adult mosquitoes collected. *Aedes aegypti* comprised 46.9% [(9,551/20,343), 5,232 female] of the total adult mosquitoes collected. One trap location was removed from the analysis due to 71.4% (15/21) of *Ae. aegypti* collections being significant outliers compared with the other collection sites in that treatment zone (Rosner's outlier test, $p < 0.05$). During the surveillance period, there was a significant effect of group on the number of adult (males + females) *Ae. aegypti* trapped in the treatment zones (12.7 ± 13.3, mean $\pm$ SD) compared with the control zone (24.9 ± 23.8, mean $\pm$ SD) (negative binomial regression, $p < 0.001$) (Figure 1).

During the 2023 adult surveillance, 418 BGS traps were set in the treatment zones and 138 in the control zone. Of these, five (four treatment, one control) were removed from the analysis due to various issues (e.g., trap failures). Three species (*Ae. aegypti*, *Cx. tarsalis*, *Cx. quinquefasciatus*) comprised $> 99.9\%$ of the total adult mosquitoes collected. *Aedes aegypti* comprised 26.3% [(11,510/43,766), 6,476 female] of the total adult mosquitoes collected. During the surveillance period, there was no significant effect (negative binomial regression, $p > 0.05$) of group on the number of *Ae. aegypti* adults (males + females) caught in the treatment zones (20.5 ± 29.3, mean $\pm$ SD) compared with the control zone (22.0 ± 30.3, mean $\pm$ SD) (Figure 2).

WALS applications resulted in a significant reduction of *Ae. aegypti* following the 2022 applications, whereas there was no significant reduction in 2023. The reduction of the *Ae. aegypti* population found in 2022 (Figure 1) matches the results of Pruszynski et al. (2017), who found a $> 50\%$ reduction of *Ae. aegypti* adults in treatment zones following aerial WALS applications. Similarly, Vetrone and Kluh (2021) found an almost 90% decline of adult *Ae. aegypti* in a treatment area following WALS applications. In 2023, our treatments were conducted before the population of *Ae. aegypti* peaked later in the summer, possibly due, at least in part, to unusual summer rainfall from the remnants of

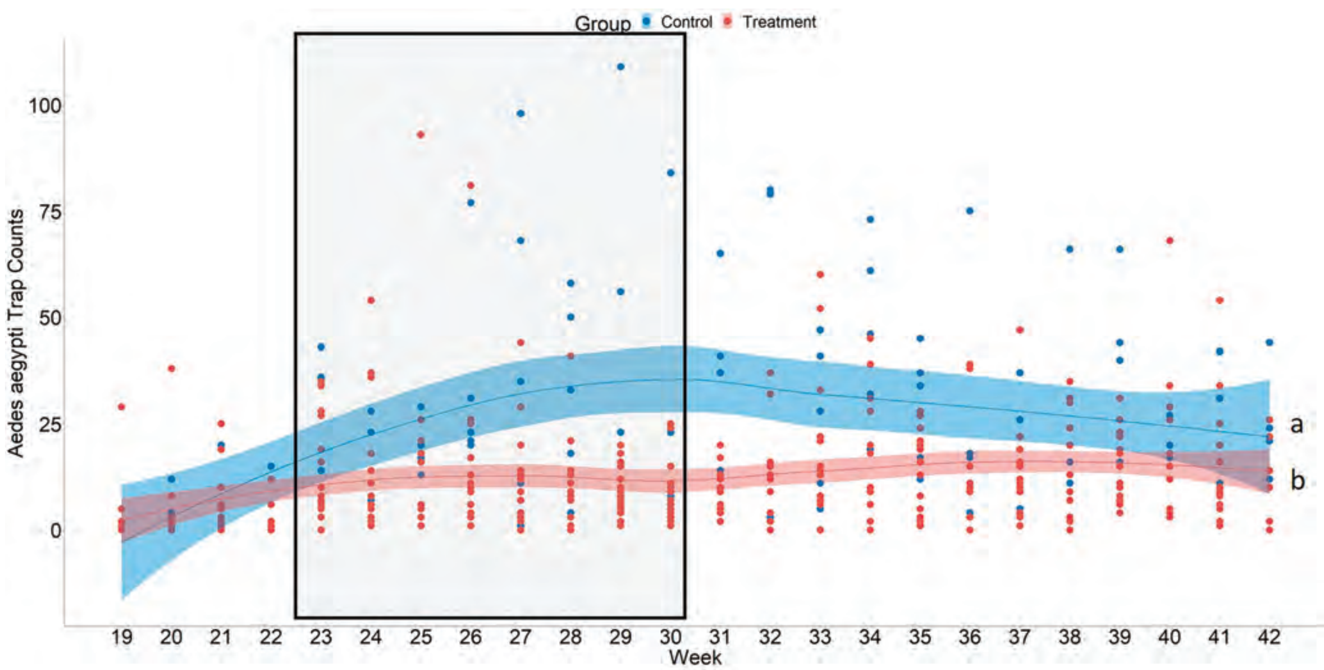

Figure 1.—Number of adult (male + female) *Aedes aegypti* collected from weekly BGS traps in control (blue) and treatment (red) zones during 2022. The boxed region indicates the period of WALS applications. Curves with different letters indicate significance (negative binomial regression, $p < 0.05$). Curves are plotted using a locally weighted polynomial regression (LOESS model). Shading shows 95% confidence intervals.

Hurricane Hilary. This midsummer addition of new sources may explain the later population increase and why we did not see a significant reduction of *Ae. aegypti* trap counts.

There was not a statistically significant ($F_{1, 154} = 0.471$, $p = 0.49$) effect of distance on larval mortality in cups placed in the front yard (79.1 ± 33.2%, n = 84) compared to those placed in the back yard (75.3 ± 33.3%, n = 74) (Figure 3). Similarly, there was no significant ($F_{(1, 154)} = 2.897$, $p = 0.09$) effect on larval percent mortality in covered (72.9 ± 34.9%, n = 80) and uncovered cups (82.0 ± 32.0%, n = 76) (Figure 3). The control group larvae experienced 6 ± 11.7% (mean ± SD, n = 28) mortality.

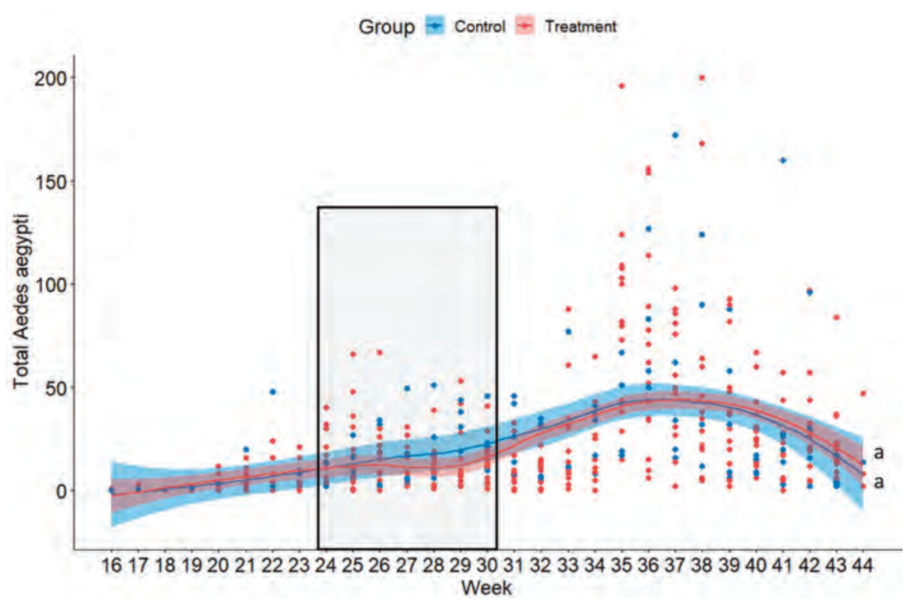

Figure 2.—Number of adult (male + female) *Aedes aegypti* collected from weekly BGS traps in control (blue) and treatment (red) zones during 2023. The boxed region indicates the period of WALS applications. Similar letters indicated no significant difference (negative binomial regression, $p < 0.05$). Curves are plotted using a locally weighted polynomial regression (LOESS model). Shading shows 95% confidence intervals.

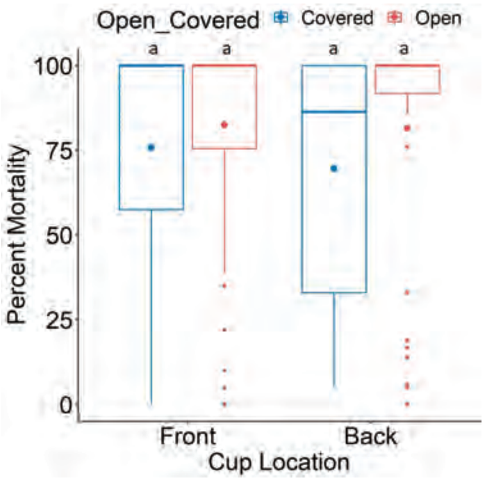

Figure 3.—Boxplots of percent mortality of larvae in covered (blue) or uncovered (red) cups placed in the front or back yards of homes following WALS applications. Similar letters indicated means were not significantly different (ANOVA, unbalanced type II, $p < 0.05$). Large points show mean value.

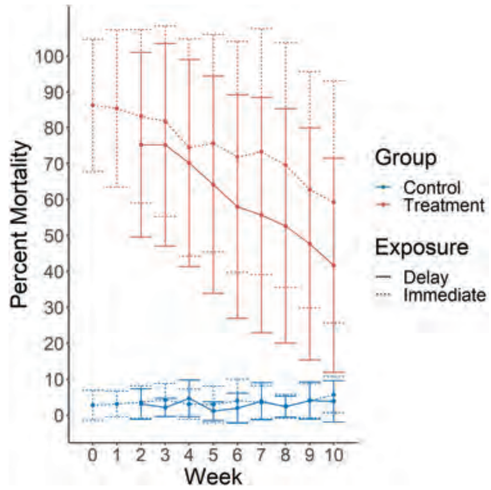

Figure 4.—Percent mortality (mean ± SD) of mosquito larvae in control (blue) and treatment (red) containers at various times post WALS application (week 0). Immediate group containers had water added upon collection, while delay group containers remained dry for two weeks post treatment.

These results correspond with other reports that WALS applications are able to reach containers in the back yards of residences or under cover such as vegetation (Pruszynski et al. 2017, Vetrone and Kluh 2021). The lack of distance effect indicated that WALS droplets were able to bypass the home and still effectively treat both the front and back yards. Morgan et al. (2021) found > 90% 24 hour larval mortality in cups placed at up to 700 ft following a cemetery WALS applications. However, in residential zones, they found decrease mortality in cups placed in backyards compared with front yards. This difference in results may be a result of fewer barriers at the cemetery site allowing for greater drift of the larvicide during WALS applications.

When the eleven weeks of mortality data was averaged for all containers, there was a significant difference (Wilcoxon rank sum test, $p < 0.001$) in the average larval percent mortality between the control (3.3 ± 4.5, mean ± SD, n = 474) and the treatment (68.2 ± 32.1, mean ± SD, n = 1440) groups during the eleven week period. Three time points (weeks 2, 5, 10) were compared among the four groups. There were no significant differences (Kruskal-Wallis, $p > 0.05$) in percent mortality in containers being tested immediately or delayed two weeks after WALS applications for any of the three time points tested (Figure 4). There was a significant difference (Kruskal-Wallis, $p < 0.05$) between the treatment and control groups for all time points (Figure 4). There was a significantly greater (Wilcoxon rank sum test, $p < 0.001$) percent larval mortality among all treatment samples at two weeks (79.2 ± 24.8%, mean ± SD, n = 144) after WALS application than at ten weeks post treatment (50.1 ± 32.8%, mean ± SD, n = 144). Morgan et al. (2021) found that mortality of larval cohorts introduced to WALS treated containers up to seven days post treatment declined in cups that were placed at > 100 ft, whereas closer cups retained high larval mortality. Because our containers were all placed in the front yards of residences, our findings of a gradual decline in larval mortality match these findings. This is likely due to containers closer to the WALS application receiving a larger amount of larvicide, and therefore retaining effectiveness for longer. We also did not find a significant difference between cups that had water added to them immediately following WALS applications compared with those that remained dry for two weeks. This shows that empty containers exposed to the larvicide prior to being filled with water may still effectively control *Ae. aegypti*. This scenario is one that is likely to occur, with dry containers filling with water (i.e. from rain, sprinklers, watering) after WALS applications. Although this experiment is an idealized test under controlled conditions, it demonstrates that the product remained effective even after remaining in a dry container that later was filled with water.

Overall, our results showed that while WALS applications are a promising method for the control of *Ae. aegypti* populations, they are dependent on the timing of application during the mosquito season. Due to their associated costs and labor requirements, treatments on the scale of an entire city are not normally feasible. However, WALS treatments can be of great benefit for small areas with abundant *Aedes* populations exploiting areas with hard to treat sources or as a larviciding component of the response to a locally transmitted case of various diseases such as dengue. While not a 'silver bullet' for mosquito control, WALS are an effective method that when utilized within the existing integrated pest management framework, can effectively reduce *Ae. aegypti* populations. Future work by the district is planned to replicate the adult monitoring experiment during 2024, as well as to evaluate local field populations to determine if there is currently any resistance to Bti present that could impact WALS effectiveness.

Conclusions

Our results show that WALS applications can have a significant impact on *Ae. aegypti* adult populations. The product was able to reach containers that were both in the front and back yards of residences, as well as those that had an obstruction such as vegetation above them. When the larval mortality in treated containers were assessed for ten weeks following WALS applications, we found that larval mortality remained high during this period. We also found that product applied to containers that remained dry for two weeks prior to the introduction of water remained capable of causing high larval mortality. Further research into how to effectively utilize WALS applications within the present control framework is warranted to improve control of this synanthropic mosquito.

Acknowledgements

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Comparative evaluation of an inexpensive mosquito trap for surveillance of invasive *Aedes* and *Culex* mosquitoes in southern California

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Abstract

Mosquito surveillance programs employ various trap designs to monitor mosquito populations and detect mosquito-borne pathogens. In California, the BG Sentinel 2 trap (BGS-2), typically baited with the BG cartridge lure and CO_2 from dry ice, has gained acceptance as the standard trapping method for assessing host-seeking populations of *Culex* and container inhabiting *Aedes* mosquitoes in urban and suburban areas. Our study evaluated the effectiveness of an economical trap constructed from a 15 L (4 gal) square black plastic bucket ("Bucket trap") compared to the BGS-2 trap for collecting *Ae. aegypti*, *Ae. notoscriptus*, and the primary West Nile virus vector, *Culex quinquefasciatus*, in Los Angeles and Orange counties. When provided with both the BG lure and CO_2, capture rates were not significantly different between the Bucket and BGS-2 traps for total mosquitoes [38.2 vs. 47.6/trap-night (TN)], *Cx. quinquefasciatus* (23.1 vs. 33.0/TN), *Ae. aegypti* (9.9 vs. 11.0/TN), and *Ae. notoscriptus* (2.2 vs. 1.8/TN). However, the BGS-2 trap caught significantly more *Cx. quinquefasciatus* females compared to the Bucket trap (32.1 vs. 18.7/TN, $P = 0.048$), whereas the Bucket trap caught significantly more *Cx. quinquefasciatus* males (4.4 vs. 0.9/TN, $P < 0.001$). When using the BG lure alone as the attractant, the Bucket trap caught significantly more *Cx. quinquefasciatus* (12.3 vs. 4.0/TN, respectively, $P = 0.009$), whereas capture rates were not significantly different between the Bucket and BGS-2 traps for *Aedes* mosquitoes. Catch sizes were notably lower without CO_2 for both traps. These findings indicate that the Bucket trap could serve as a cost-effective alternative to the BGS-2 trap for collecting mosquitoes of medical importance.

Introduction

Mosquito-borne arboviruses, such as dengue, Zika, chikungunya, and yellow fever, pose significant public health threats, especially in urban settings where dense human populations facilitate rapid viral spread (Gubler 2011). Effective surveillance and control of mosquito populations are essential for preventing outbreaks of diseases caused by these viruses. *Aedes aegypti* (L.) is the main urban vector of dengue, Zika, chikungunya, and yellow fever viruses (CDC 2018), and members of the *Culex pipiens* complex, *Cx. pipiens* L. and *Cx. quinquefasciatus* Say, are key vectors for West Nile virus in urban and suburban regions of the U.S. (Andreadis 2012). Traditional surveillance methods, such as human landing counts, ovitraps, CO_2-baited CDC-style traps (Sudia and Chamberlain 1962), and gravid traps, have been employed to monitor their populations, along with those of other mosquitoes. However, the efficacy of these methods varies based on trap design, location, and the targeted species. Numerous studies have shown that CO_2-baited, CDC-style traps are not very effective in capturing *Ae. aegypti* and *Ae. albopictus* (Skuse) (Li et al. 2016, CDC 2018) and *Cx. pipiens* complex mosquitoes in urban areas (Resien et al. 1999, Li et al. 2016).

Advancements in mosquito trap technology have led to the development of traps specifically engineered to target medically-important, container inhabiting *Aedes* mosquitoes. One innovation is the 12 V DC Biogents Sentinel 2 trap (BGS-2), which has become widely accepted as the most efficient trap for monitoring the abundance of *Ae. aegypti* and *Ae. albopictus* (Kröckel et al. 2006, CDC 2018). The BGS-2 trap employs a counter-airflow design principal similar in concept to Kline et al. (1999), with the trap entrance ("capture zone") positioned at the center and surrounded by an attractant plume of CO_2 and the scent of the BG lure dispersed around the perimeter of the capture zone (Biogents 2024). This arrangement draws mosquitoes closer to the entrance for collection in a net. Despite its improved efficiency compared to other traps, studies show the BGS-2 catches only a relatively small fraction of host-seeking *Aedes aegypti*, possibly due to the mosquito's flight behavior in evading the capture zone (Amos et al. 2019, Amos et al. 2020). The BGS-2 trap is relatively costly (ca. $160/unit) and exhibits low durability after repeated use in field conditions (RC, pers. obs.).

In response to these limitations of existing traps, we developed a novel "Bucket trap" (Figure 1) and field-tested this design in several urban settings in Los Angeles and Orange Counties. The trap consists of a 15 L (4 gal) square

Figure 1.—The Bucket trap showing the collection chamber (A) and fan motor (B) secured in position by an elastic strap (C) fastened across the underside of the bucket lid (D).

black plastic bucket, an ABS inlet tube, an insulated container for dry ice, a 12 V DC battery-powered computer fan motor to generate airflow, and a modified plastic food storage container serving as the collection chamber (i.e., "net"). The Bucket trap also incorporates a counter-airflow design concept and can be fabricated with hand-powered tools and readily available materials. The trap can be used with a BG cartridge lure alone, or with the BG lure combined with CO_2, as attractants. We discuss trap design features, configuration of the attractants, and deployment strategies for enhancing mosquito capture rates in urban environments.

Materials and Methods

Bucket trap construction

Five square-shaped 15 L (4 gal) black plastic buckets (Uline Model S-13650BL), equipped with lids (Uline Model S-13651BL), were modified into traps. Each bucket had a 127 mm (5 in.) dia. hole drilled approximately 76 mm (3 in.) below its top rim on all four sides. A 78 mm (3.0625 in.) dia. hole was drilled at the center of each lid, along with multiple 3 mm (0.125 in.) holes spaced evenly around the perimeter of the lid encircling the center hole as part of the counter-airflow design principle.

The collection chamber was constructed from a 1.9-liter (2 qt.) square plastic food storage container (KaTom Restaurant Supply in Kodak, TN). Two 102 mm (4 in.) dia. holes were drilled into the container, one on the lid and the other on the bottom. A wire mesh screen was affixed over the lid hole to retain the catch, and an elastic fabric sleeve was fitted onto the bottom of the container. Powering the Bucket trap was a 12V DC water-resistant computer fan motor measuring 120 mm × 120 mm × 25 mm (CoolerGuys Model CG12025H12B2-3Y) and was placed onto the screened lid of the collection chamber to induce airflow. On the underside of the bucket lid, a 30 cm (11 in.) long elastic strap was attached at opposite sides for the purpose of holding the collection chamber and motor in place (Figure 1).

A 3 in. (76 mm) internal dia. ABS tube, measuring 115 mm (4.5 in.) in length, functioned as the inlet tube through which air entered the trap. One end of this inlet tube was inserted into a 3-in. ABS hub × hub coupler fitting. The coupler served to extend the height and width of the trap entrance and to hold the inlet tube securely on top of the bucket lid. The combination of the inlet tube and coupler was then mounted on the bucket lid and inserted through the center hole into the collection chamber.

An insulated foam container [Uline Model S-20447, 1.3 L (0.35 gal capacity)] was used to hold dry ice. To allow for the release of CO_2 gas as the dry ice sublimated, two small holes [5 mm (0.25 in. dia.)] were drilled into opposite ends of the foam container. The overall material cost of the trap was approximately $75 US.

BGS-2 and Bucket trap set up and operation

Bucket trap set up: Prior to deployment to the field, the insulated container was filled with approximately 1 kg (2.2 lbs.) of dry ice pellets and placed in the bottom of the bucket. Subsequently, the bucket lid was turned upside down atop the bucket. The collection chamber was attached to the underside of the bucket lid using the elastic strap. The 12V DC fan motor was then secured to the collection chamber lid by the strap (Fig. 1). The bucket lid, with the assembled collection chamber and motor unit, was then restored to its upright position, placing the collection chamber inside the bucket. The bucket lid was seated loosely in place on top of the bucket for easy removal. Once the bucket lid was in position, the lower end of the inlet tube, with the coupler on its upper end, was inserted through the lid hole and into the collection chamber. The BG cartridge lure was positioned atop the dry ice container. Finally, the collection chamber's sleeve was drawn through the lid hole and tightened around the inlet tube (Fig. 2).

Bucket trap operation: The motor is started by connecting the wires to a 12 V DC 12 amp-hr. battery positioned beside the trap. Once under power, air is drawn through the inlet tube into the collection chamber and expelled through the fan motor inside the bucket. The exhausted air mixes with the scent of the BG lure and CO_2 emitted from the insulated container and is blown through the four large holes in the sides of the bucket and upward through the small holes in the bucket lid, following the counter-airflow principle. Upon trap

Figure 2.—Configuration of the Bucket trap: A) inlet tube, B) bucket lid seated on bucket, C) collection chamber, D) computer fan motor, E) BG cartridge lure, and F) insulated container.

retrieval, the inlet tube is removed while the motor is running to ensure that the mosquitoes remain in the collection chamber. The sleeve to the collection chamber is then closed to secure the catch, after which the motor is disconnected from the battery. The bucket lid/collection chamber/motor combination is then disassembled.

BGS-2 trap set up and operation: BGS-2 traps were configured according to the manufacturer's guidelines (Biogents 2024). The BGS-2 trap was baited with dry ice as was the Bucket trap, with the insulated container inside the BGS-2 trap.

Study sites and experimental design

BGS-2 and Bucket traps were placed at five study sites in Los Angeles County (2 coastal sites in the cities of El Segundo and Los Angeles near L.A. International Airport) and Orange County (3 inland sites in the cities of Anaheim and Fullerton) from 1 July–22 November 2023. The sites consisted of a several suburban habitats including three residential properties (El Segundo, Los Angeles, and Fullerton), a churchyard (Fullerton), and a cemetery (Anaheim). The number of traps deployed varied by site, ranging from 1–2 trap pairs (BGS-2/Bucket traps) on residential properties to four trap pairs on the larger churchyard and cemetery properties. Traps were set midmorning on shaded ground next to vegetation, spaced at least 10 m (33 ft.) apart, and operated for approximately 24 hrs. until retrieval. To prevent positional bias, trap types were rotated the following day among locations within each study site and resupplied with dry ice, empty collection chambers and fully-charged 12 V batteries. During a specified week, all traps were configured with the same attractants, either the BG lure only or a combination of the BG lure and dry ice. Weather data were obtained from weather stations located at Los Angeles International Airport for the L.A. County sites and Fullerton Municipal Airport for the Orange County sites (Weather Underground, website: https://www.wunderground.com/).

Specimen processing and data analysis

After trap retrieval, mosquitoes were held on dry ice until processing in the laboratory. Mosquitoes were identified by species and sex using taxonomic keys, enumerated, and recorded. Mosquito abundance was determined by calculating the average number per trap-night (avg./TN). The number of trap-nights varied by study site, depending on the number of traps deployed at a particular location. Data were analyzed for normality, and the non-parametric Wilcoxon rank sum test was used for significance testing. A p-value < 0.05 was used for statistical significance. No comparative analysis was conducted across sites concerning species abundance, distribution, or diversity.

Results

BG lure only

During trap comparison trials employing only the BG lure, a total of 1,096 mosquitoes representing five species were captured from 30 July–3 November in 50 trap-nights (Table 1, A). The number of trap-nights ranged from 4–26 per study site (Table 2). *Culex quinquefasciatus* was collected most frequently, accounting for 68.3% (n = 200/293) and 76.7% (n = 616/803) in the BGS-2 and Bucket traps, respectively. This was followed by *Ae. aegypti* at 23.2% (n = 68/293) and 16.8% (n = 135/803), and *Ae. notoscriptus* (Skuse) at 8.5% (n = 25/293) and 5.9% (n = 47/803) for the BGS-2 and Bucket traps, respectively. The remaining collections comprised small quantities of *Culiseta incidens* (Thompson) (n = 3) and *Cx. tarsalis* Coquillett (n = 2).

Significantly more mosquitoes were collected in the Bucket trap compared to the BGS-2 trap (16.1 vs. 5.9/TN, respectively, $P < 0.05$) (Fig. 3, A). For *Cx. quinquefasciatus*, the Bucket trap collected significantly more mosquitoes compared to the BGS-2 trap (12.3 vs. 4.0/TN, respectively, $P < 0.001$, Fig. 3, B), along with more females (4.2 vs. 1.8/TN, respectively, $P = 0.009$, Fig. 3, C) and more males (8.1 vs. 2.2/TN, respectively, $P < 0.001$, Fig. 3, D). The Bucket and BGS-2 traps yielded statistically equivalent numbers of *Ae. aegypti* and *Ae. notoscriptus* (Fig. 3), and no significant differences in counts were observed between the sexes of these two *Aedes* species in the BGS-2 and Bucket traps (Table 1, A).

Table 1.—Mosquito collections by species from BGS-2 and Bucket traps, July – November, 2023, at five study sites in Los Angeles and Orange counties, A) BG lure only. B) BG lure + CO_2.

	A) BGS Lure Only (50 Trap-Nights)						B) BGS Lure + CO_2 (66 Trap-Nights)					
	Male		Female		Total		Male		Female		Total	
Species	BGS	Bukt	BGS	Bukt	BGS	Bukt	BGS	Bukt	BGS	Bukt	BGS	Bukt
Cx. quinquefasciatus	108	406	92	210	200	616	58	291	2122	1232	2180	1523
Ae. aegypti	14	63	54	72	68	135	281	328	442	327	723	655
Ae. notoscriptus	1	1	24	46	25	47	1	4	116	143	117	147
Cs. incidens	0	0	0	3	0	3	0	5	79	154	79	159
Cx. stigmatosoma	0	0	0	0	0	0	0	1	16	11	16	12
Cx. tarsalis	0	0	0	2	0	2	0	1	14	10	14	11
Cs. particeps	0	0	0	0	0	0	0	1	5	5	5	6
Cs. inornata	0	0	0	0	0	0	0	0	8	9	8	9
Ae. taeniorhynchus	0	0	0	0	0	0	0	0	1	1	1	1
Totals	**123**	**470**	**170**	**333**	**293**	**803**	**340**	**631**	**2803**	**1892**	**3143**	**2523**
Avg/TN	2.5	9.4	3.4	6.7	5.9	16.1	5.2	9.6	42.5	28.7	47.6	38.2

BG lure + CO_2

During trap comparison trials employing the BG lure + CO_2 combination, a total of 5,666 mosquitoes representing nine species, were collected from 1 July–22 November in 66 trap-nights (Table 1, B). The number of trap-nights varied from 4 - 26 per study site (Table 2). *Culex quinquefasciatus* accounted for the highest proportion of captures, comprising 69.4% (n = 2,180/3,143) and 60.4% (n = 1,524/2,523) for the BGS-2 and Bucket traps, respectively, followed by *Ae. aegypti* at 23.0% (n = 723/3,143) and 26.0% (n = 655/2,523), and *Ae. notoscriptus* at 2.5% (n = 79/3,143) and 5.8% (n = 147/2,523), respectively. Other species included *Culiseta incidens*, *Cx. tarsalis*, *Cx. stigmatosoma* Dyar, *Cs. inornata* (Williston), *Cs. particeps* (Adams), and *Ae. taeniorhynchus* (Wiedemann) (Table 1, B).

The BGS-2 trap caught significantly more *Cx. quinquefasciatus* females compared to the Bucket trap (32.1 vs. 18.7/TN, respectively, $P = 0.048$, Fig. 4, A), whereas the Bucket trap caught significantly more *Cx. quinquefasciatus* males compared to the BGS-2 trap (4.4 vs. 0.9/TN, respectively, $P < 0.001$, Fig. 4, B). Both the BGS-2 and Bucket traps yielded statistically similar numbers of total mosquitoes for *Cx. quinquefasciatus* (47.6 vs. 38.2/TN, respectively), *Ae. aegypti* (11.0 vs. 9.9/TN, respectively), and *Ae. notoscriptus* (1.8 vs. 2.2 avg./TN, respectively) (Fig. 4). Furthermore, there were no significant differences between the BGS-2 and Bucket traps in the counts by sex for these two *Aedes* species (Table 1, B).

Weather data

Between July and November 2023, the average daily temperature was approximately 19.4°C (66.9°F) along the coastal sites of Los Angeles County, with temperatures ranging from 10°–31.7°C (50.0 – 89.1°F). For the inland Orange County locations, the average daily temperature was approximately 21.3°C (71.3° F), with temperatures ranging from 8.9°C–35.6°C (48.0 – 96.1°F). Average wind speeds remained below 8 km/hr. (5 mph) during mosquito collection periods. On August 21, the subtropical storm Hillary brought about 64 mm (2.5 in.) of rainfall to the region.

Table 2.—Mosquito numbers by species and trap type at each study site in Los Angeles and Orange Counties, July – November 2023. Mosquitoes were collected in BGS-2 and Bucket traps using the BG cartridge lure and CO_2 as attractants.

	El Segundo Residence		LA City Residence		Fullerton Residence		Fullerton Church		Anaheim Cemetery	
Trap-Nights	26		4		12		10		14	
Species	BGS	Bukt	BGS	Bukt	BGS	Bukt	BGS	Bukt	BGS	Bukt
Cx. quinquefasciatus	1,840	1,176	83	202	86	44	111	77	60	25
Ae. aegypti	0	1	20	42	154	115	258	268	291	229
Ae. notoscriptus	110	136	7	11	0	0	0	0	0	0
Cs. incidens	50	129	26	27	3	1	0	1	0	1
Cx. stigmatosoma	9	8	2	2	1	1	1	0	3	1
Cx. tarsalis	7	6	3	2	1	0	0	1	3	2
Cs. particeps	1	1	4	4	0	0	0	0	0	0
Cs. inornata	4	5	4	4	0	0	0	0	0	0
Ae. taeniorhynchus	1	1	0	0	0	0	0	0	0	0
Totals	**2,022**	**1,463**	**149**	**294**	**245**	**161**	**370**	**347**	**357**	**258**
Avg/TN	77.8	56.3	37.3	73.5	20.4	13.4	37.0	34.7	25.5	18.4

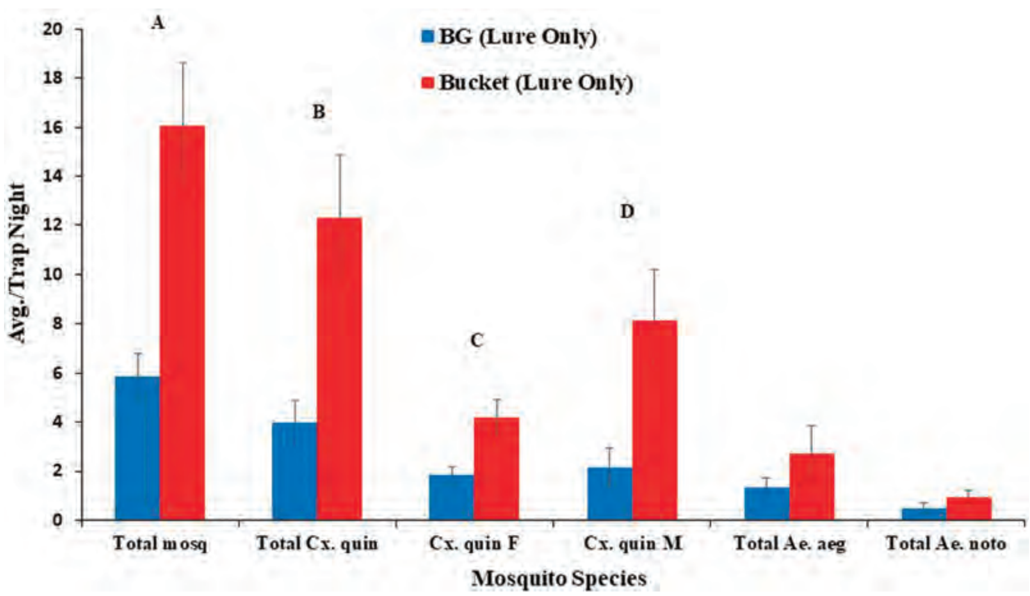

Figure 3.—Average number of total mosquitoes (mosq.) per trap-night by species (Cx. quin. = *Cx. quinquefasciatus*; Ae. aeg. = *Ae. aegypti*; Ae. noto. = *Ae. notoscriptus*; F = females and M = males) in BGS-2 and Bucket traps using the BG lure only as attractant. (A) $P < 0.001$ (B) $P = 0.009$ (C) $P < 0.001$ (D) $P = 0.004$. Vertical bars represent mean ± SE. Unless indicated, differences between the two traps are not significant.

Discussion

The study involved a comparative analysis of two mosquito trap designs for their efficacy in capturing mosquitoes. The primary objective was to explore the viability of creating a cost-effective mosquito trap that matched the performance of the commercially available BGS-2 trap. This investigation took place during the warm summer and fall seasons in southern California, coinciding with peak mosquito abundance. Test sites were situated in densely populated residential areas with suitable avian and mammalian hosts, abundant vegetation, and peridomestic aquatic habitats conducive to container inhabiting mosquitoes (Schreiber et al. 1989, Reisen et al. 1990, Cummings 1992, Krueger et al. 2015). On August 20–21, the subtropical storm Hillary inundated the region with

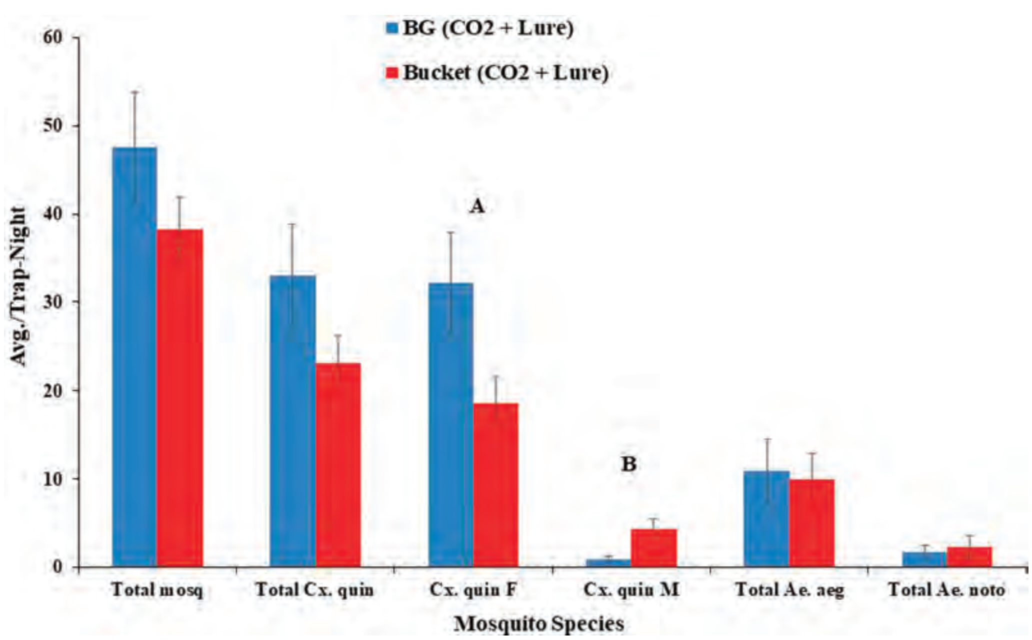

Figure 4.—Average number of mosquitoes per trap-night by species (abbreviations follow Fig. 3) collected in BGS-2 and Bucket traps using the BG lure + CO_2 combination. (A) $P = 0.048$ (B) $P < 0.001$. Vertical bars represent mean ± SE. Unless indicated, differences between the two traps are not significant.

rainfall, further contributing to the presence of water in these habitats.

In general, the Bucket trap demonstrated comparable effectiveness with the BGS-2 trap in capturing *Cx. quinquefasciatus*, *Ae. aegypti*, *Ae. notoscriptus*, and other mosquito species. However, when utilizing the BG lure with CO_2, the BGS-2 trap captured more female *Cx. quinquefasciatus* than the Bucket trap (Fig. 4), whereas the capture rates of the Bucket trap for male *Cx. quinquefasciatus* and *Ae. aegypti* were consistently higher compared to the BGS-2 trap, regardless of the attractant used (Table 1).

The inclusion of CO_2 increased mosquito capture rates and species diversity for both traps compared to using the BG lure alone (Table 1). Carbon dioxide, when combined with the BG lure, synergistically increased the collection of host-seeking females in each trap by several times compared to using the BG lure alone. However, more male *Cx. quinquefasciatus* were captured in the Bucket trap using only the BG lure compared to the BG lure with CO_2, 8.1 vs. 4.4/TN, respectively. The reasons for this disparity are unclear. Because traps with different attractant arrangements were not simultaneously used at the same locations, no direct comparisons for significance could be drawn from these data. However, these results align with previous trap studies, indicating that incorporating CO_2 with the BG lure enhances catch sizes and species diversity for the BGS-2 trap (Ferreira de Ázara et al. 2013).

Culex quinquefasciatus and *Ae. aegypti* were the predominant species collected across all locations, with the highest numbers recorded at the residential property in El Segundo and the Fullerton church site, respectively (Table 2). *Aedes notoscriptus* was detected only at the two coastal locations in Los Angeles, in the general area where this species is reported to have its highest abundance in California (Metzger et al. 2021). Small quantities of *Cx. stigmatosoma*, *Cx. tarsalis*, *Cs. incidens*, *Cs. particeps*, and *Ae. taeniorhynchus* were captured at some or all of the study sites (Table 2). In general, mosquito species diversity was greater in the coastal sites in Los Angeles County compared to the inland locations in Orange County (nine vs. five species, respectively) (Table 2). All mosquito species collected in the study are known to feed on humans, and several (*Culex* spp. and *Ae. aegypti*) are competent arboviral vectors.

We used a 15 L (4 gal) square black bucket with a matching black lid for our trap design, although other bucket sizes, shapes, colors, and attractant configurations are available (Zhu et al. 2019). In future studies, we plan to explore using white lids in combination with dark-colored buckets (such as black or blue) to enhance color contrast, a known feature for attracting host-seeking *Ae. aegypti* (Fay and Prince 1970). The square shape of the Bucket trap, along with its sturdy design, facilitated carrying trap components (collection chamber, insulated dry ice container, motor, lure, and battery) within each bucket prior to deployment. This design also allowed for compact storage of traps during transportation and easier placement at the sites, in contrast to the fabric-framed, collapsible BGS-2 trap, which required set up in the field.

Figure 5.—Bucket trap modified into a gravid trap by placing a Bundt cake pan (A) filled with attractant media for mosquito oviposition over trap inlet tube.

The Bucket trap's cost-effectiveness and ease of fabrication make it a viable option for widespread use in settings with limited resources. Additionally, the Bucket trap's heightened attractiveness to male mosquitoes, possibly due to its semi-gloss, black color (Fay and Prince 1970), suggests that it has potential utility for assessing the effectiveness of "rear and release" programs involving sterile, genetically-modified, or *Wolbachia*-infected male mosquitoes for suppressing populations of *Cx. quinquefasciatus*, *Ae. aegypti,* and *Ae. albopictus*.

Conclusion

Effective mosquito surveillance requires accurate measurement of adult mosquito abundance, and advancements in trap design can enhance surveillance capabilities. The Bucket mosquito trap was developed to be cost-effective, easy to fabricate in-house with commercially-available components, and durable. Furthermore, the trap's configuration allows for adaptation with a suitably-sized Bundt cake pan mounted over the inlet tube, enabling its use as a gravid trap (Fig. 5). Further studies are needed to optimize this design for dual use as a host-seeking and gravid trap, particularly for enhancing the capture rate of *Ae.*

aegypti, to improve mosquito monitoring and disease prevention efforts.

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Set it, forget it, regret it: The creation and management of a catch basin larvicide efficacy monitoring program

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Introduction

The Catch Basin Program at the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) has observed a decline in the residual effectiveness of larvicide products containing the active ingredients s-methoprene and spinosad in Woodland, CA catch basins (Wheeler and Wagner, 2022). Additionally, larvicide products used by SYMVCD routinely fail to provide residual control consistent with stated label efficacy ranges within catch basin habitats. This marked departure from the label-stated residual life of larvicide products has prompted close monitoring for both residual life and the development of resistance to preserve the effectiveness of these active ingredients. Previous sampling efforts involving either comparisons of pre-treatment and post-treatment larval abundance or on-site emergence inhibition monitoring were ad hoc and limited in scope. To better monitor the residual activity and suitability of different larvicides for catch basin mosquito control, a standardized season long protocol was developed for monitoring multiple catch basins across Sacramento and Yolo Counties.

Methods

To better quantify the residual duration of larvicides used at SYMVCD, specific catch basins were identified as study sites across the SYMVCD service area. Because a wide range of factors influence the residual action of larvicide products, study sites included a minimum of ten catch basins and were spread across multiple cities and neighborhoods. Larvicides observed to determine the residual effective period included Altosid XR-B (s-methoprene), Altosid Pellets WSP (s-methoprene), and Sumilarv 0.5G (pyriproxyfen).

To assess residual activity, catch basins were treated with designated larvicides and then sampled weekly to determine the level of control. For basins treated with s-methoprene and pyriproxyfen, pupae were collected and observed for emergence inhibition (EI) using the petri dish method. Briefly, a 100X15mm polystyrene petri dish was lined with two 4-6 cup basket-type coffee filters that were trimmed to fit within the petri dish. A film of water from the respective basin collected concurrently with the pupae was pipetted into the bottom of the dish to completely saturate the coffee filter but not flood the dish. Pupae were placed on the damp filter paper, the lid replaced, and the pupae observed for emergence. Untreated pupae were included in each assay as handling controls.

In addition to collecting pupae, the immature mosquito population from each test basin was assessed by larval dipping. Four dips were collected, and the total number of each instar and pupae were counted and averaged across the four dips (data not presented here). A threshold of $<70\%$ EI for three consecutive sampling events was used as the criterion to end the monitoring period. In addition, basins were removed from the study when they could not be reliably accessed (e.g. a vehicle was parked on a catch basin grate, or a grate had become immovable.).

Results and Discussion

SYMVCD catch basin treatment schedules were designed to limit the number of times active ingredients were applied to a given basin in a calendar year. The treatment schedule predominantly relied on the usage of long-acting larvicides formulated with s-methoprene, spinosad, and pyriproxyfen, targeting a single use of active ingredient per calendar year and *Bacillus thuringiensis israelensis* formulations when additional treatments were required.

A successful rotation is contingent on retreatment periods. Collection of data on residual life of available products is ongoing, but the data collected in 2022 and 2023 on Altosid XR-B (s-methoprene), Altosid Pellets WSP (s-methoprene), Sumilarv 0.5G (pyriproxyfen), and Duplex-G (s-methoprene, bti.) are shown in Figure 1. Emergence inhibition drifting above and below the 70% EI threshold as observed in the basins treated with Altosid Pellets WSP and Altosid XR-B was common. Several factors that may have contributed to measurement variation included constantly changing water levels, flushing of the basins by large water flow events, and inconsistent collection of pupae. Despite the presence of larvae, pupal numbers were at times unexpectedly low. Because only pupae were collected for emergence inhibition testing, this directly impacted study

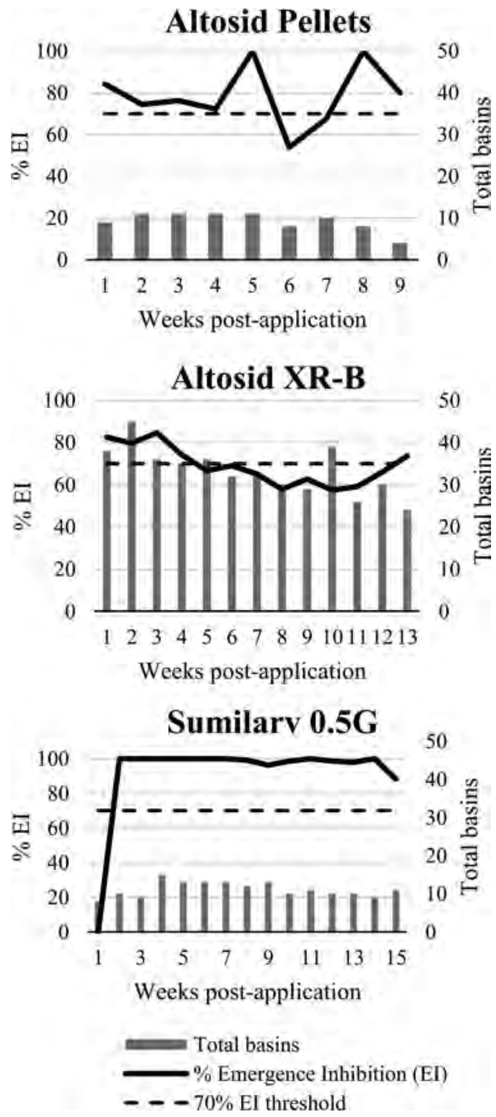

Figure 1.—Emergence inhibition (EI) by weeks post-treatment for three different larvicides applied to catch basin habitats (Altosid Pellets, Altosid XR-B, and Sumilarv 0.5G). The total number of basins observed at each time point are shown in the vertical bars and the % EI for collected pupae is represented by the black line. For reference the dotted line represents the 70% EI threshold for re-treatment.

outcomes. Low pupal numbers likely were due to larvicide activity that led to early pupal death.

The addition of Sumilarv 0.5g to the catch basin product rotation has allowed a single long-term application once per year. At a rate of 10g per basin this product has provided up to 15 weeks of consistent control (Figure 1). The implementation of a continuous monitoring program has allowed for fine scale adjustments of retreatment intervals and product rotation schedules. These combine to provide more consistent and sustainable management of these widespread and difficult-to-control aquatic mosquito habitats.

Conclusion

The paradigm shift from limited or arbitrary monitoring of the residual activity of larvicides used in catch basins to widescale testing has provided insight into product performance in the field. Changes in larvicide effectiveness - including potential larvicide resistance issues - were apparent and sourced from data accumulated in a consistent manner. These data were used to inform retreatment intervals and rotation schedules.

Acknowledgements

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Assessing the ability of Planet Laboratories' SuperDove satellites to detect unmaintained swimming pools as potential larval habitats for West Nile virus vectors

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Introduction

Neglected swimming pools have been identified as important urban mosquito sources (Reisen et al., 2008), and eliminating mosquito production from neglected swimming pools is a primary focus of mosquito control programs in California. Effective control of mosquitoes in swimming pools relies on chemical treatments such as chlorine and filtration of water that eliminates mosquito eggs and larvae. During warmer seasons when alternative oviposition sites are scarce, swimming pools lacking filtration systems, circulation pumps, and sanitation measures can become ideal habitats for mosquitoes.

In recent years, the integration of remotely sensed imagery with GIS technologies, along with mosquito surveillance data, has contributed significantly to the detection of potential mosquito habitats in both urban and non-urban settings (e.g., Kim et al., 2011; Thompson et al., 2013). However, on-demand technologies and limited revisiting frequencies of orbiting satellites or airborne devices result in image acquisition only at specific points or infrequent intervals, hindering reliable detection of changes in swimming pool conditions for mosquito control. Recent advances in satellite technology now offer higher temporal resolution, enabling more frequent imaging of the Earth at weekly or daily intervals while maintaining high spatial resolution.

Our study investigated the potential use of high spatial and temporal resolution satellite imagery for the early detection of changes in swimming pool conditions. Specifically, we sought to compare spectral data between neglected pools with algal growth and well-maintained pools in Fresno city and neighboring areas in California's Central Valley.

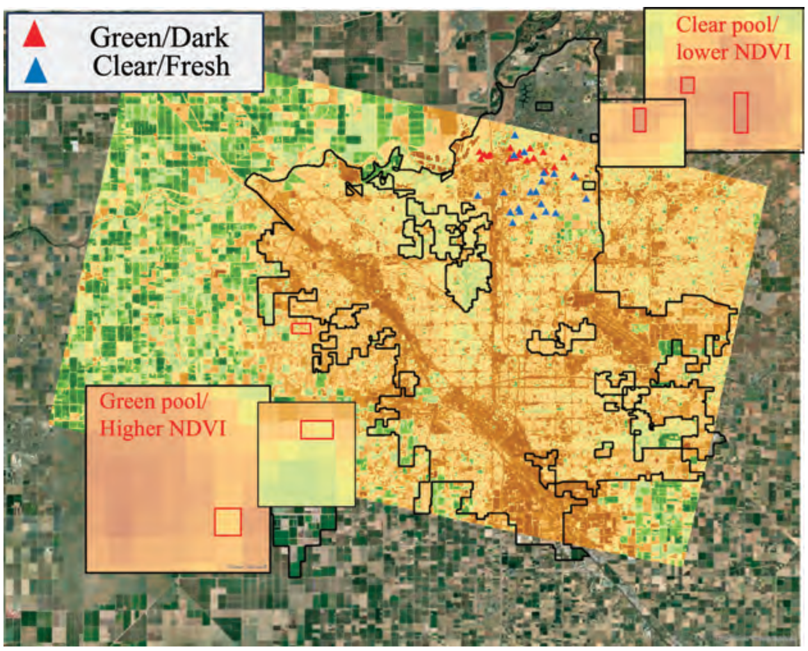

Figure 1.—The figure illustrates the locations of clear swimming pools (depicted as blue triangles) and dark/green swimming pools (depicted as red triangles). The true-color satellite image of the study area serves as the background, while the overlaid square layer represents NDVI values (derived from PlanetScope ortho-scenes) on June 14th, 2022. Two insets provide examples of the NDVI signature of a dark/green swimming pool (bottom-left) and a clear swimming pool (top-right), both marked in red.

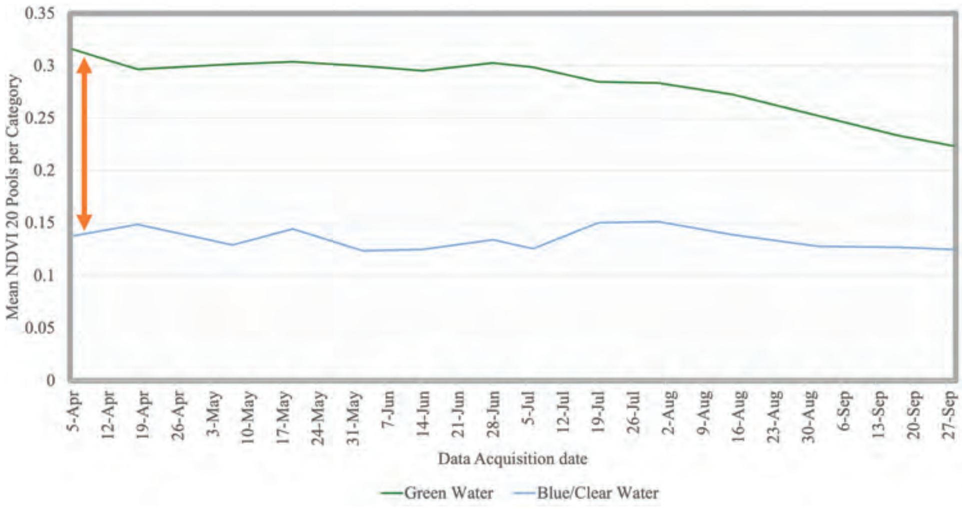

Figure 2.—Smoothed trends (lines) representing sets of 20 dark/green swimming pools (depicted in blue) and clear/fresh swimming pools (depicted in green) from April to September 2022.

Methods

A data set provided by the Consolidated Mosquito Abatement District (CMAD) documented the locations and detailed the condition of pools inspected by the district from 2017 to 2023. We specifically targeted 20 pools that consistently exhibited a green hue, indicative of poor maintenance and algal growth, during two consecutive inspections in 2022. Additionally, we identified 20 pools that consistently maintained a blue hue, indicative of proper maintenance and clear water, during the same period (Figure 1). We employed daily satellite imagery from the PlanetScope satellite constellation (Planet Labs, California, USA) to analyze the normalized difference vegetation index (NDVI). We obtained 35 cloud-free multispectral raster tiles corresponding to 35 dates from the PlanetScope dataset during the 5 Apr – 27 Sep 2022 study period. These tiles were utilized to calculate the NDVI values for the 40 swimming pools covering the hottest part of the year in Fresno.

Results

Neglected swimming pools with algal growth (“green pools”) exhibited significantly higher NDVI values compared to well-maintained “blue pools” with clear water (Welch’s *t*-test, $t = 19.723$, $P < 0.001$), providing evidence for a potential method for distinguishing between neglected and clean pools using PlanetScope imagery (Figure 2). The direction of the difference was consistent throughout the 2022 study period, although there was a gradual decrease in NDVI values of neglected pools later in the study period, while NDVI values for clean pools remained stable. This decrease in NDVI among neglected pools may be linked to factors such as chemical treatments applied by pool owners as the season progressed or diminished algal growth later in summer (Figure 2).

Conclusion

Effective utilization of PlanetScope’s NDVI has proven successful in distinguishing variations in greenness between neglected and well-maintained swimming pools. Correlations were established between higher NDVI values and the neglected state of swimming pools, as confirmed by inspections by CMAD. Leveraging high-frequency temporal data from PlanetScope, we can continuously monitor the condition of swimming pools throughout the year and document transitions in water clarity, from green to clear or vice versa. Moving forward, several key steps are planned. First, we will employ an object-oriented methodology to segment urban land use, specifically to identify swimming pools. Second, we will broaden the scope of the study to encompass new indices alongside NDVI, thereby analyzing a larger sample set of swimming pools and potentially enabling more detailed characterization of pool conditions. Finally, we will explore longitudinal use of daily imagery over time to swiftly identify changes in swimming pool conditions, particularly the shift from clear blue to green or dark green hues, indicative of neglect.

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Aerial and ground larvicide applications for *Aedes aegypti* control in the Coachella Valley

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Introduction

In 2023, the Coachella Valley Mosquito and Vector Control District (District) conducted aerial and ground Wide Area Larvicide Spray (WALS) operations in the cities of Rancho Mirage and Palm Springs. These applications required extensive planning and close collaboration among District staff and other stakeholders. The primary objective of these efforts was to reduce the emergence of adult *Aedes aegypti*, thereby reducing the potential transmission of introduced viruses. The planning processincluded mosquito surveillance and monitoring to identify high-risk areas, selecting larvicides based on their efficacy and environmental safety, and utilizing specialized ground and aerial application equipment to ensure thorough coverage. Importantly, the District worked closely with local authorities, and community stakeholders to ensure transparency and prioritize safety throughout the execution of these mosquito control measures.

Methods

To determine the areas targeted for control, the District collected and reviewed *Ae. aegypti* adult trap collection data, larvae samples and the number of mosquito service calls from 2022. This was done to determine the target areas. Utilizing available resources, a maximum of 1,200 acres were selected for truck mounted application, and 1,700 acres for helicopter applications. Utilizing Arc GIS mapping software, one thousand acre hexagons were created that contained data from trap collections and larvae and pupae collections. The hexagons were color coded to rank areas based on *Ae. aegypti* abundance (Figure 1). Based on these findings, both Palm Springs and Rancho Mirage were determined to have the highest adult and larval collections. The City of Rancho Mirage was divided into two routes, with each assigned an A1 Super Duty equipped with Micronair atomizer application equipment manufactured by A1 Mist Sprayers, and a pilot truck (Figure 2). A Monitor LT was used to record the areas where the applications were performed (Figure 2). VectoBac® WDG was applied at 0.25 lbs. per acre at a mixed ratio of 1:1. Equipment flow rate was set at 1.5 gallons per minute, with an application speed of 10 mph and a swath of 300 feet. Aerial application was done in the City of Palm Springs by Ocean Air Services at the application rate of 0.25 lbs. of VectoBac® WDG per acre, with an application speed of 60 mph and a swath of 125 feet.

Results and Discussion

The District planned for a total of eight applications to be done at 0200h on six consecutive Saturdays starting on 22 Jul 2023 and every other Saturday for the last two applications. There was one cancellation on 19 Aug due to

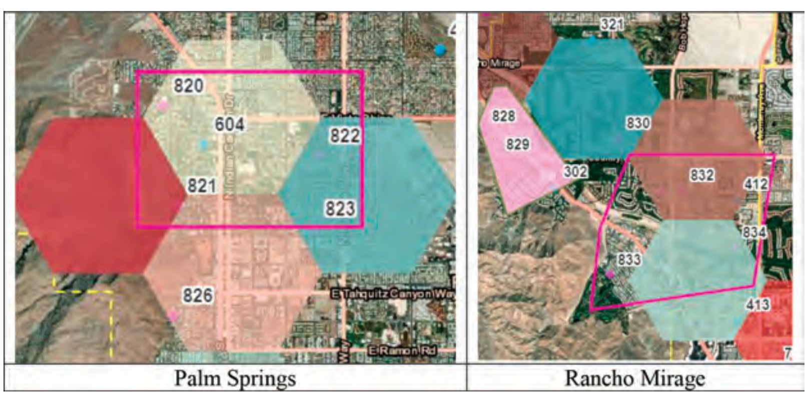

Figure 1.—ESRI Map illustrating hexagons with high *Aedes aegypti* populations. Red hexagon illustrates highest adult and larval collections.

Figure 2.—A1 Super Duty used to conduct truck mounted larvicide applications. Monitor LT was used to record data on the application area.

Figure 3.—Venturi equipment used for mixing the control product.

inclement weather from Hurricane Hilary. The District covered 1,208 acres per mission in the City of Rancho Mirage and 1,700 acres per mission in the City of Palm Springs. An important part of the planned applications was the complete mixing of the control product to avoid clumping or improper mix ratios. To accomplish this in collaboration with shop staff, a mixing station the included a venturi process was built in-house and used to mix the needed product for the ground applications (Figure 3). Mixing required 2 hrs. and was done the day before application.

Figure 4.—Map illustrating an application done 26 Aug 23 in the City of Rancho Mirage. Green outline signifies area sprayed spray, red outline signifies no application.

Conclusion

The Wide Area Larvicide Spray program was accomplished through meticulous planning, strategic collaboration, and innovative mixing techniques, Utilizing specialized ground and aerial application equipment ensured that all planned missions were carried out efficiently across the targeted areas (Figure 4). Collaboration among CVMVCD departments, local authorities and community stakeholders has fostered transparency, communication, and community engagement in mosquito control efforts.

Acknowledgements

The Coachella Valley Mosquito and Vector Control District acknowledges the shop staff and operations crew which assisted with set up, mixing and mission applications, in addition to the Laboratory, Information Technology, Public Outreach and Operations departments for assisting with the planning and community outreach of the targeted locations.

First known report of an infestation of bird fleas (*Dasypsyllus gallinulae perpinnatus*) in a condominium in the San Francisco Bay Area**

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**A complete version previously was submitted for publication to the Journal of Medical Entomology

Introduction

The bird flea, *Dasypsyllus gallinulae perpinnatus,* is a common parasite in passerine bird nests on the western coast of the United States (Holland 1985). Although it may be regularly identified by ornithologists and flea taxonomists, it is rarely reported by pest control operators and vector control technicians even in incidental collections from unmanaged open space areas or parks.

In March 2023, the San Mateo County Mosquito and Vector Control District investigated a condominium property in South San Francisco, CA for a reported a flea infestation inside the residence. The resident was collecting an average of seven fleas per week from both the upstairs and downstairs of the condominium for a period of two weeks. They had two dogs who were on oral flea medication (NexGuard/afoxolaner) and were walked locally through the neighborhoods. Only one member of the household reported receiving bites.

Methods and Results

The front walkway, back yard, and garage were flagged for fleas using a 1-meter square white flannel cloth. A total of 31 fleas (13 males, 18 females) were collected from the entryway of the house, one flea was collected from the backyard, and 20 (9 males, 11 females) were collected by the resident from inside the house. A small abandoned bird nest was discovered and removed from a fire alarm bell in the front walkway. From this nest, a total of 387 fleas (163 males, 224 females) were recovered from the nesting material and all life stages (egg, larvae, pupae, and adult) were observed within it. The presence of bird lice and larval western black-legged ticks (*Ixodes pacificus*) also were noted. The moveable process of all male fleas (a diagnostic feature) was examined under a dissecting microscope and a subset of fleas were slide mounted to confirm the species identification. All examined male fleas were identified as *D. g. perpinnatus*.

Conclusions

Although *D. g. perpinnatus* is a common parasite of birds, we are not aware of any records of structural infestations by this species. It is possible the lack of reports may be due to a lack of knowledge or willingness to collect, slide-mount, and identify fleas by pest control operators. However, given that many flea species have host associations or preferences (Lewis et al. 1988), the identification of the flea can assist in determination of the host animal, which is a necessary part of creating an integrated pest management plan.

We encourage our colleagues in vector control and the broader pest control community to continue to develop and use accessible reference materials for the routine identification of fleas. The control of bird fleas may necessitate different techniques than would be employed for controlling mammal-associated fleas. As many mammal-parasitizing fleas are effective zoonotic disease vectors (Bitam et al. 2010), the possibility that *D. g. perpinnatus* may be capable of transmitting avian pathogens to humans should not be discounted. Research should be conducted to determine the vector competency and infection prevalence of *D. g. perpinnatus* with flea-borne zoonotic pathogens.

Acknowledgements

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Efficacy of Amdro® Pro and Extinguish® Plus against the red imported fire ant (RIFA), *Solenopsis invicta*, in Orange County, CA

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Abstract

The Orange County Mosquito and Vector Control District (District) uses several granular insecticidal baits to combat infestations of the red imported fire ant (RIFA), *Solenopsis invicta.* The program employs a modified “Texas-Two Step” treatment strategy, with a primary mound treatment followed by a broadcast treatment of granular bait with a different mode of action. From July to November 2023, the District conducted a study on RIFA control at a 19-ha (47-acre) cemetery in central Orange County. The objective was to evaluate the long-term effectiveness of two fire ant baits, Amdro® Pro and Extinguish® Plus, in reducing RIFA populations across four treatment zones using low [1.12 kg/ha (1.0 lb./acre)] or high [1.68 kg/ha (1.5 lbs./acre)] label application rates. The treatment zones varied in size from 0.45 to 2.03 ha (1.10–5.01 acres). To assess ant activity before and after treatment, five monitoring plots were established within each zone. Ten small plastic mesh baskets baited with corn chips were attached to survey flags arranged in a circle with a radius of 4.6 m (15 ft.) in each evaluation plot and left in the field for 60 min before retrieval. Post-treatment assessments occurred during weeks 1, 2, 4, 8, 12, and 16 following the initial evaluation (week 0). Four weeks after treatment, applications of Amdro Pro and Extinguish Plus at 1.68 kg/ha (1.5 lbs./acre) showed reductions of 43.4% and 75.5%, respectively, in RIFA abundance compared to pre-application data; however, only the reduction by Extinguish Plus was significant ($P = 0.003$). Although Extinguish Plus at 1.68 kg/ha (1.5 lbs./acre) outperformed Amdro Pro at the same rate in suppressing RIFA populations initially, neither product sustained significant reductions in RIFA abundance beyond four weeks at all application rates. By the midpoint of the study (8 weeks), RIFA populations had rebounded to or exceeded pre-treatment levels, gradually decreasing with the cooler weather by the study’s end at 16 weeks. Based on these findings, the District intends to continue utilizing the higher 1.68 kg/ha (1.5 lbs./acre) application rate, coupled with timely applications of products with different modes of action, to effectively manage RIFA populations in Orange County.

Introduction

The red imported fire ant (RIFA), *Solenopsis invicta* Buren, is an invasive and aggressive agricultural and public health pest (Jemal and Martin 1993). RIFA arrived from South America into the United States in the 1930s near Mobile, Alabama, and have become established throughout the southern and southwestern regions of the U.S. (Vinson 1997, Greenberg et al. 1999). The introduction of RIFA into California has been traced to a shipment of beehives from Texas in 1997 after which they colonized almond orchards in Kern County (CDFA 1998). In 1999, RIFA were discovered in a nursery and several residential areas in Orange County (CDFA 2000, Spitzer 2000). RIFA colonies subsequently have been found across all cities in Orange County, with notable RIFA hotspots in the northeast and southwest regions (Figure 1). Because of their impact on humans, the Orange County Mosquito and Vector Control District (District) has been examining ways to control RIFA since their discovery in the county (Nelson et al. 2013, Greenberg et al. 2015).

The District’s RIFA program consists of two main workflows, one addressing RIFA infestations in large areas such as schools, parks, greenbelts, cemeteries, and golf courses, and the other focusing on infestations at single-family residences. The District’s residential RIFA control program involves collaboration among District staff and residents on abatement efforts at single-family properties (Nelson et al. 2013, Greenberg et al. 2015). The District’s large-area RIFA control program employs a modified “Texas-Two Step” treatment strategy using granular fire ant baits, with a primary mound treatment followed by successive broadcast treatments (Drees and Merchant 2006). In this strategy, Siesta® (BASF Corporation, Research Triangle Park, NC) (active ingredient 0.063% metaflumizone, a sodium channel blocker) is initially applied to active mounds for rapid knockdown of stinging workers. Within a week, an Amdro® Pro (BASF) (active

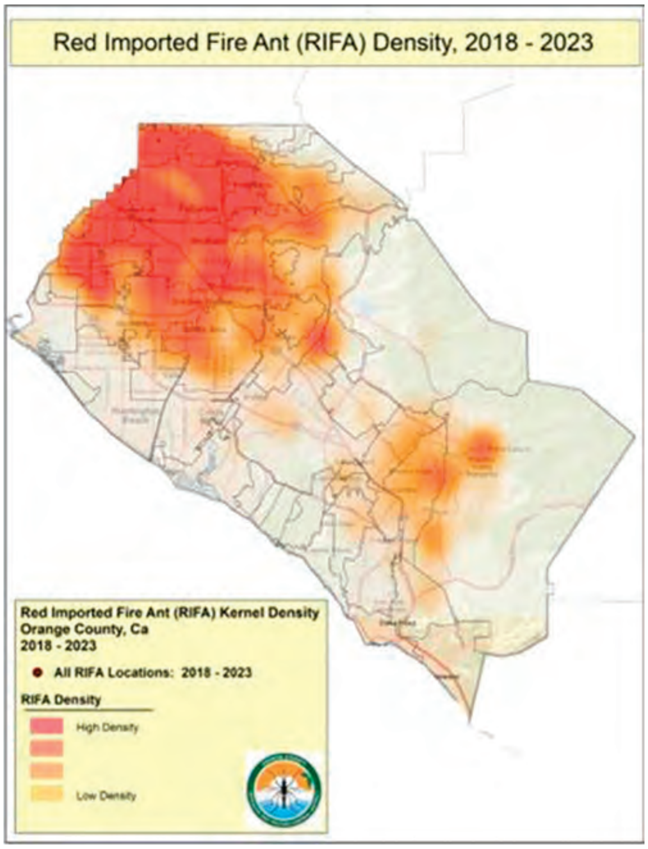

Figure 1.—RIFA hotspot map, Orange County, California, 2018–2023.

ingredient 0.73% hydramethylnon, a metabolic inhibitor) broadcast is administered to ensure longer-term control. This is further complemented by an Extinguish® Plus (active ingredients 0.25% S-methoprene, an insect growth regulator, and 0.365% hydramethylnon) broadcast after 90 days. As a final step, an Esteem® (Valent USA, LLC, Walnut Creek, CA) (active ingredient 0.5% pyriproxyfen, an insect growth regular) broadcast treatment is applied after an additional 90 days. In routine pesticide applications for RIFA control, the District typically administers treatments at the highest recommended label rates for the available products. The current study compared the efficacy and residual activity of two RIFA baits, Amdro Pro and Extinguish Plus, when applied at both their lowest [1.12 kg/ha (1.0 lbs./acre)] and their highest [1.68 kg/ha (1.5 lbs./acre)] label rates. Building upon previous research on Siesta and Advion® at various application rates (Perezchica-Harvey and Henke 2016), our study also included a cross-comparison of products to determine the most effective product and application rate for achieving significant reductions in RIFA populations.

Methods

Specific criteria were used to select appropriate study sites: 1) The site needed to be larger than 1.62 ha (4 acres) to accommodate four treatment zones with a minimum buffer distance of 39.6 m (130 ft.) between evaluation plots within each zone. This buffer distance was determined based on previous research indicating a maximum RIFA foraging distance of 17 m (56 ft.) (Stringer et al. 2011). 2) RIFA populations were established throughout the site. 3) The site should not have received a prior broadcast pesticide treatment in 2023. 4) The site must allow for the undisturbed placement of baited flag baskets in the same locations to monitor RIFA colony abundance at defined time points. 5) Schools or public parks were excluded from consideration. Based on these criteria, the 19 ha (47-acre) Holy Sepulcher Cemetery in Orange, California, was chosen as the location for the 16-week study. The site allowed open entry for District employees but limited access to the public. Regular irrigation of the cemetery turf created an ideal habitat for RIFA growth and proliferation.

Pre-application data were collected on 25 July 2023 to establish baseline RIFA abundance at the cemetery. Treatment and monitoring were performed mid-morning when RIFA were most active. District staff mapped RIFA mounds scattered throughout the cemetery, identifying areas with the highest density of above-ground mounds. The eastern section of the cemetery was partitioned into four distinct zones. Two northern zones were designated for Extinguish Plus applications, while the two southern zones were allocated for Amdro Pro applications. Zones were sequentially labeled from one to four, spanning from north to south. On 26 July, three licensed applicators used hand spreaders to apply the pesticide granules evenly at the specified application rates. The application rates and products were administered as follows: Zone One received Extinguish Plus at a rate of 1.68 kg/ha, Zone Two received Extinguish Plus at 1.12 kg/ha, Zone Three received Amdro Pro at 1.12 kg/ha, and Zone Four received Amdro Pro at 1.68 kg/ha. Owing to concerns regarding RIFA abundance to workers and patrons of the cemetery, a non-treated control area was omitted from the study. The remaining area of the cemetery underwent an application of Amdro Pro at a rate of 1.68 kg/ha.

Each zone contained five replicated evaluation plots, ranging in size from 0.45–2.03 ha (1.10–5.01 acres), with a minimum buffer distance of 39.6 m (130 ft.) maintained between them to mitigate potential interference (Stringer et al. 2011). Evaluation plot centroids were established based on the highest mound abundance within each zone. Each plot was provided with ten survey marking flags baited with salted corn chips (Frito® Lay/PepsiCo, Plano, TX) arranged in a circle within a 4.6 m (15 ft.) radius (Fig. 2). These flags, each attached to a 4 cm dia. x 4 cm high plastic mesh basket filled with corn chips, were easily accessible to ants through ground contact (RIFA are readily attracted to the oils on corn chips). A total of 200 flags were deployed and retrieved each collection week, with the geolocation of each evaluation plot centroid carefully logged to ensure accurate flag placement during subsequent collection weeks. Flags were retrieved after 60 min in the field, after which their baskets were removed and placed in uniquely-labelled plastic bags. Specimens were held on dry ice and processed in the District laboratory. Ants were identified using

Figure 2.—Aerial photo of one of four treatment zones in Holy Sepulcher Cemetery. Each zone contained five circular evaluation plots with ten equally-spaced survey flags/buckets baited with salted corn chips.

taxonomic keys, enumerated, and recorded. RIFA abundance was assessed based on the number of ants collected per basket per hour of collection and averaged for a treatment zone (50 baskets per zone).

Post-treatment assessments were conducted from July to November 2023 at weeks 1, 2, 4, 8, 12, and 16, culminating with the final collection on 14 November. Statistical analysis was performed using a generalized linear model (GLM) with a binomial distribution and logit link function in R (v4.2.3) to ascertain the significance ($P < 0.05$) of Amdro Pro and Extinguish Plus at both 1.12 kg/ha and 1.68 kg/ha application rates for residual efficacy at weeks 4 and 8 only. A cross-comparison of product efficacy was also performed.

Weather Data

Weather data for the Holy Sepulcher Cemetery was obtained from a meteorological recording station in the city of Orange via the website World®WeatherOnline.

Results and Discussion

During the 16-week study, a total of 53,313 RIFA were collected, along with 110 ants belonging to various other species, which were excluded from the data analysis. Although the study site was monitored for 16 weeks, analysis of RIFA abundance focused solely on weeks 1, 2, and 4 post-treatment, after which RIFA abundance rebounded to or exceeded pre-treatment levels by week 8. This indicated that the effectiveness of both products had waned shortly after the initial four weeks of the study. RIFA abundance dropped below baseline levels from mid-October to November (weeks 12 and 16, respectively), coinciding with cooler weather (Fig. 3 and Fig. 4). Furthermore, evaluating the efficacy of RIFA insecticidal bait treatments beyond the initial four weeks would lack validity without data from a comparable non-treatment control zone within the cemetery.

Both application rates of Amdro Pro resulted in declines in average abundance during weeks 1, 2, and 4 compared to pre-treatment data (Fig. 3). Post-treatment, RIFA abundance experienced reductions at week 1 (−57.3 and −77.4%), week 2 (−13.3 and −32.8%), and week 4 (−37.3 and −43.4%) for the 1.12 kg/ha and 1.68 kg/ha applications, respectively. There was no significant difference between the two application rates in the GLM for Amdro Pro.

Weekly results for Extinguish Plus varied based on the application rate. Extinguish Plus at 1.68 kg/ha resulted in a decrease in RIFA abundance compared to baseline data during weeks 1, 2, and 4 (−77.4, −11.2, and −75.5%, respectively), whereas Extinguish Plus at 1.12 kg/ha exhibited increases of up to five times pre-treatment data

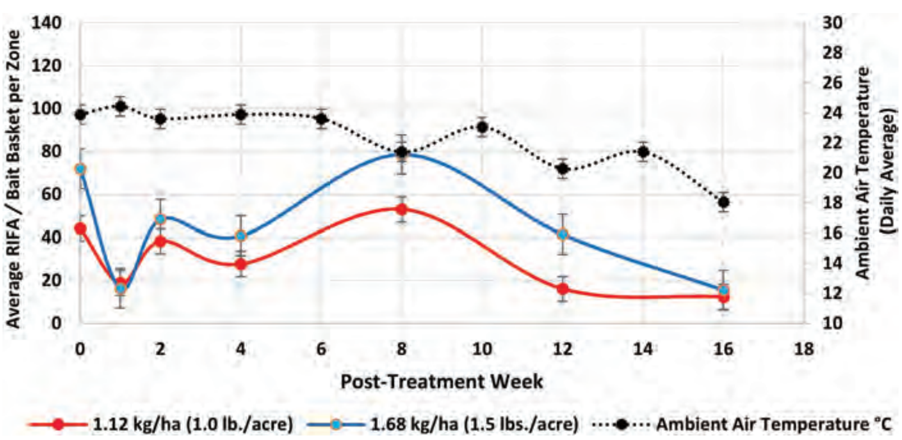

Figure 3.—Efficacy of Amdro Pro at different application rates. Vertical bars represent mean ± SE.

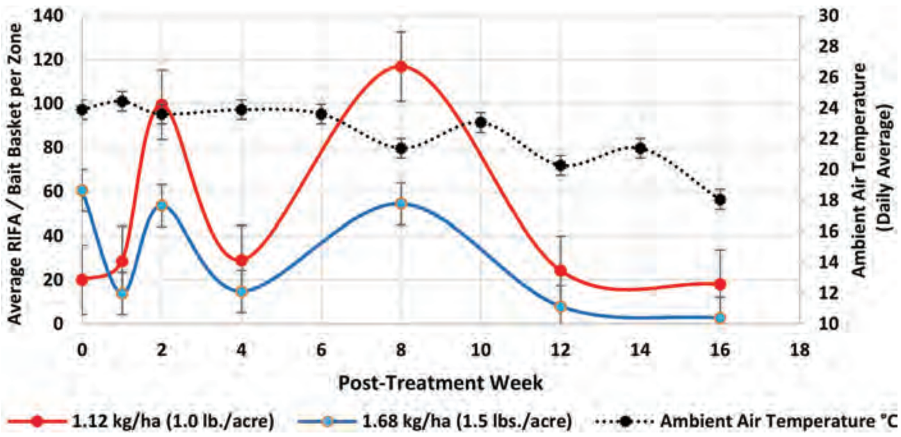

Figure 4.—Efficacy of Extinguish Plus at different application rates. Vertical bars represent mean ± SE.

(Fig. 4). A significant reduction was observed at week 4 (−75.5%, $P = 0.003$) for the 1.68 kg/ha treatment. When comparing Extinguish Plus at 1.68 kg/ha to Amdro Pro at the same rate, Extinguish Plus showed a significantly better performance in reducing average RIFA abundance at week 4 −75.5% vs. −43.4% for Extinguish Plus ($p < 0.046$).

The increases in RIFA abundance at week 2 compared to week 1 for both application rates of Amdro Pro and the higher Extinguish Plus treatment were attributed to the emergence of ants from pupae that were unaffected by the applications. The reasons behind the increase in RIFA abundance in the zone treated with the 1.12 kg/ha Extinguish Plus application (Zone 2) remain unclear. It was possible that this zone might have inadvertently received irrigation immediately after the RIFA treatment, leading to inadequate consumption of the bait by the foraging ants, which prefer dry bait.

The average daily temperature in central Orange County averaged around 21.3°C (71.8°F) from July to mid-November 2023 (Figures 3 and 4). In general, the warm daily temperatures were conducive to optimal activity for RIFA from July–October. The lowest temperature and daily average [10°C (50°F); 18.1°C (64.5°F)] occurred on the last collection day, 14 November, and may have affected RIFA activity on that particular day. Porter and Tschinkel (1987) reported a decrease in RIFA foraging activity when temperatures fell below 20°C (68°F). In total, approximately 23 mm (0.90 in.) of rainfall was recorded during the study period, with most (21 mm; 0.83 in.) falling on August 20–21 from subtropical storm Hillary (World®WeatherOnline 2023).

Our study investigated the effectiveness and residual activity of one-time broadcast applications of two insecticidal baits to control RIFA at an irrigated cemetery. Amdro Pro, which contains the metabolic inhibitor hydramethylnon, quickly kills RIFA larvae and adults, reducing the immediate stinging risk to the public, with control purportedly lasting 2 to 6 months (TIFARMP 2024). In contrast, Extinguish Plus combines hydramethylnon with the IGR S-methoprene, which not only kills ants directly, but also sterilizes surviving queens through the action of the IGR. This dual-action approach is considered effective, purportedly providing control for 2 to 12 months (TIFARMP 2024). To enhance efficacy, an optimal version of the Texas Two-Step treatment protocol recommends first applying a broadcast treatment of an IGR to ensure it is fed to the queen before applying Amdro Pro, or similar fast-acting products, directly to mounds to eradicate the workers. Additionally, Greenberg et al. (2015) noted that relying solely on Amdro Pro applications on residential properties led to RIFA treatment failures of up to 33%, unless followed within a month by an IGR treatment. In an earlier study similar to ours, Perezchica-Harvey and Henke (2016) found that sustained RIFA control was achieved only with the higher application rate (1.68 kg/ha) of Advion granular bait at irrigated parks in the Coachella Valley.

Conclusion

Only Extinguish Plus, containing two active ingredients, exhibited a significant decrease in average RIFA abundance at 4 weeks post-treatment compared to pre-application data when applied at the higher rate of 1.68 kg/ha. However, no product demonstrated significant reductions in RIFA abundance beyond the initial four weeks at any application rate. Although no discrepancy was noted between the application rates of 1.68 kg/ha and 1.12 kg/ha for Amdro Pro, a greater decrease in RIFA abundance was observed with the higher rate of 1.68 kg/ha over the four-week period. Based on these results, the District intends to continue using the higher application rate of 1.68 kg/ha to effectively manage RIFA populations in Orange County.

Since their discovery in Orange County in 1999, RIFA have become established throughout the county. Managing them effectively requires a comprehensive strategy that includes multiple treatments using several insecticides with different modes of action, varying application techniques, and carefully timed schedules. Such measures are essential in reducing the risk of re-infestation at treated sites. Further investigations focusing on the operational efficacy of the District's modified Texas-Two Step RIFA treatment protocol may provide insights into the overall effectiveness of different application rates and represent the next step in evaluating the success of the District's ongoing RIFA control program.

Acknowledgements

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To Scout and shield: The formation, function, and future of the invasive mosquito control program at the Sacramento-Yolo Mosquito and Vector Control District

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Introduction

The detection of the urban-adapted invasive mosquitoes, *Aedes aegypti,* in the city of Citrus Heights (California, USA) in September 2019, and *Aedes albopictus* in Carmichael, a suburb of Sacramento (California, USA) in 2022, marked the beginning of a critical and ongoing challenge for the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD). The absence of a dedicated program for managing these invasive species presented an unsustainable burden on existing SYMVCD departments. To address the potential number of mosquito development sites across our jurisdiction's 465,000 residences, efforts were undertaken to establish surveillance protocols, develop new forms of public outreach, and implement Bti-based (*Bacillus thuringiensis israelensis*) wide-area larvicide spraying (WALS).

To address the challenges presented by these two invasive species in Sacramento and Yolo Counties, a dedicated Urban Operations Program was created by staff and approved by the SYMVCD Board of Trustees. The establishment of the Urban Operations Program required adapting existing surveillance and control tools to operate effectively at a more localized level and targeted a wide range of human-made sources across an urban landscape. Here, we highlight the formation and evolution of the SYMVCD Urban Operations Program and our efforts to address the need for the effective control of *Ae. aegypti* and *Ae. albopictus*.

Methods

Mapping

Initially, SYMVCD used paper-based maps to track detections and plan work-flow events. However, the system was inefficient due to the high volume of residential parcels within District boundaries and the lack of dynamicity. Previous district mapping efforts consisted of a paper map, containing colored coded "site" parcels, and did not offer any functionality to monitor actions, such as trapping, positive detections, or treatments. The first step to improve our system was to integrate the "whiteboard" software, Miro (Miro, San Francisco, CA, USA). Miro allowed everyone to view the paper map online and to make comments on the map in real time. Although actions still had to be double entered into the existing data management platform, technicians were able to use and update the map from the field. Miro provided a digital bridge, but the goal was to create a standalone mobile application that incorporated all required components into one platform. This was accomplished using AppSheet (Google, Seattle, Washington, USA), a mobile application that used map-based data collection. This app served as a communication tool for technicians, a data repository for actions conducted in the field, and a portal for looking at previous actions and data.

Public Outreach

A vital component of controlling *Ae. aegypti* and *Ae. albopictus* is public interaction and engagement. The number of properties, compared to staff available to perform inspections, prompted an reassessment of public outreach tools. The goal was to use effective messaging to target specific neighborhoods where invasive *Aedes* were detected. New outreach measures included targeted mailers containing information about future inspections, invasive species, and steps for reducing larval development sites. In addition to door-to-door inspections, local media coverage via live news segments, print media, social media engagement, and advertisements.

Surveillance

Currently, the gold standard for invasive *Aedes* surveillance is the BG Sentinel trap (BG-S, Biogents, Regensburg, Germany) set with BG Lure and dry ice (carbon dioxide) as bait. Prior to the creation of the Urban Operation Program, BG-S were deployed for invasive *Aedes* surveillance, but not to the extent that they were used post-detection. Currently trapping efforts for invasive *Aedes* focus both on monitoring areas of known infestation and as a mechanism to detect new infestations. In areas with previous detections, BG-S traps are set at a trap density of 5–10 traps per 1.5–3.0 sq mi. In areas without previous detections, BG-S traps are set at a density of 10 traps per 5.0 sq mi. All BG-S traps are set with both dry ice and BG lure and operated for one trap night. Trap densities are dependent on housing density, other trapping efforts, previous detections, and the state of the infestations. Early detections required higher trap densities to define the infestation area. Ultimately, the Urban Operation Program aims to implement a grid system to create more consistent and defined criteria for WALS and ultra-low volume (ULV) treatments. Overall, in 2023, the district set 2,351 BG-S traps in both Sacramento and Yolo Counties for invasive *Aedes* surveillance.

Wide Area Larvicide Spraying

Because *Ae. aegypti* and *Ae. albopictus* utilize a wide variety of containers for larval development within the urban landscape, targeting each of these sources is extremely difficult. To combat this challenge wide-area larvicide spraying (WALS; Valent Biosciences, Libertyville, Il, USA) has been implemented. To do this a A1 mist blower (A1 mist sprayers, Ponca, NE, USA) was used to spray small droplets of Vectobac WDG (active ingredient *Bacillus thuringiensis israelensis*; Valent Biosciences, Libertyville, Il, USA) across the urban landscape so that it settled into cryptic sources and targeted developing larvae. These applications are made early in the morning to avoid pedestrians. In 2023, SYMVCD conducted WALS across 9,300 acres in response to invasive *Aedes* detections. In addition to the wide area applications made with A1 mist blowers, smaller scale WALS applications were made using Stihl backpack mist blowers (Stihl, Waiblingen, Germany).

Results and Discussion

The adoption of digital mapping tools and the AppSheet mobile application has enabled efficient monitoring of technician actions as well as mosquito activity in real-time, thus improving our operational capabilities. District supervisors are able to make informed decisions for early morning WALS treatments and were better able to direct surveillance and control efforts. This has eliminated the need for paper maps which can be time consuming to create and are ineffective and cumbersome. Intensified public outreach efforts contributed to increased community engagement and awareness about invasive mosquito management. The implementation of widespread use of BG-S traps allowed for monitoring of known infestations and detection of new areas of establishment. Integration of WALS has provided a method for targeting a whole neighborhood and not just one house at a time, increasing our ability to scale up control interventions. Finally, control technicians served as the backbone of the program, conducting surveillance, yard inspections, public outreach, and control interventions.

Conclusions

The establishment of the Urban Operations Program at SYMVCD represents a proactive approach to managing invasive mosquitoes in urban landscapes. Despite ongoing challenges such as the need for additional staff resources and the need for enhanced community interaction, the program continues to evolve and adapt. Future initiatives include, refining action thresholds for WALS treatments, forecasting staffing needs, and expanding public outreach efforts to high-infestation areas.

Acknowledgements

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Evaluating mosquito control strategies: methods and challenges

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Introduction

Vector control agencies are tasked with the challenge of protecting the public from vector-borne disease and nuisance. Different agencies face a range of vector species such as rats, fleas, ticks, and mosquitoes. The Sacramento-Yolo Mosquito and Vector Control District utilizes an Integrated Vector Management (IVM) approach for managing mosquito populations (Lizzi et al., 2014) that includes public outreach and education, mosquito surveillance, biological control, larval habitat management, microbial and chemical control of aquatic life stages, and finally adult control. The approach to control is hierarchical and starts by targeting the immature mosquito stages before they emerge as adults. However, there is still a need to target adult mosquitoes to reduce nuisance biting or to interrupt transmission cycles, especially the transmission of West Nile virus by *Culex pipiens* and *Culex tarsalis*. There are a range of strategies that are utilized to control different mosquito species and life stages. Some of these strategies are well established and others are new to the field of vector control, but all require periodical assessment to ensure expected results are attained.

Here assessment strategies were assembled by level determined by the type of effect measured. Level 1 strategies measure the effect of an active ingredient or formulated product in the laboratory where both the pesticide exposure method and mosquito are controlled. Level 2 strategies measure the effect of a pesticide application on sentinel mosquitoes in the field, and level 3 strategies measure the effect of a pesticide application on the targeted mosquito population. The following is an overview of the types of assessments that can be performed at each level and some considerations for their usage.

Assessment strategies by level

Level 1 strategies generally consist of bioassays. The most common bioassay for adult mosquitoes performed in the United States is the Center for Disease Control and Prevention Bottle Bioassay (McAllister and Scott, 2019). The CDC bottle bioassay is a susceptibility assay that measures time to mosquito mortality following exposure to a diagnostic dose. This assay provides insight into whether a specific active ingredient in a formulated product is effective against a specific mosquito population. The bottle bioassay can be modified by adding inhibitors that block the expression of detoxifying enzymes to identify resistance mechanisms. The bottle bioassay also can be converted into an intensity bioassay (Lenhart et al., 2024) that compares mortality in mosquitoes at 1, 2, 5, and 10 times the diagnostic dose to quantify resistance intensity.

To evaluate larvae populations a cup bioassay can be used to assess levels of larvicide resistance (World Health Organization, 2005). This assay differs from the CDC bottle bioassay that typically uses one diagnostic does and measures time to mortality. The larval cup assay exposes larvae to multiple doses so that lethal doses (LD_{50} and LD_{90}) can be calculated. The LD_{50} and LD_{90} of both susceptible and field populations are used to calculate resistance ratios (Keiding, 1976) between susceptible and field populations that can be used to describe levels of resistance.

Level 2 strategies evaluate field applications using controlled or caged mosquito populations and are referred to as semi-field trials. One of the most commonly used examples is an adult mosquito sentinel cage trial. These types of trials are generally used to assess ultra-low volume (ULV) space sprays designed to control adult mosquitoes. Depending on the application type an array of enclosures (sentinel cages) that hold mosquitoes of known or unknown susceptibility are placed in the spray path and are used to determine whether field applications are effective. These trials benefit greatly from inclusion of both target field populations and known susceptible controls. The susceptible populations provide confirmation that the space spray reached the cage array (all susceptible dead), and the response of the field population indicates whether the selected product is effective against the targeted field population. If mortality in susceptible controls is less than 100%, it indicates that a cage array received a sub-optimal application of insecticide. This could be attributed to wind, cage orientation, or issues with application equipment. Deploying droplet impingers at each cage array can provide additional information about the droplet spectrum that passed by cage arrays and whether droplets were within product label specification.

Level 2 strategies also are available for testing larvicides. One example is the use of cups placed for collection of larvicide droplets following wide area larvicide sprays (WALS). These cups may be oriented in transects to measure effective swath width or placed throughout treatment areas to assess how larvicide droplets are deposited across a treatment area. The cups are placed in the field dry and are collected after the application. Cups are returned to the laboratory where water, larvae and larval food are added to the cups. The larvae are then observed over a period of time for mortality. Untreated controls are included as handling or negative controls. Percent mortality for each cup should be theoretically correlated with the amount of product that was deposited in the cup, where 0% mortality equates to no deposition and 100% mortality indicates a sufficient level of deposition. Values that fall in between represent the dynamic range of the test.

Level 3 strategies measure the effect of an application on the targeted field population. One approach is to measure percent reduction in either adult and larval populations following ULV or larvicide applications. This is done through pre- and post-application trapping of adults or dipping of immature stages within both treatment and matched control areas. Percent reduction is calculated using Mulla's formula (Mulla et al., 1971). Additionally, statistical models can be used to evaluate the overall impact of control strategies (Holcomb et al., 2021), but individual applications can not be analyzed using this strategy.

Conclusion

There is a continual need to assess the performance of mosquito control interventions against target populations. This is important both for developing new techniques and for evaluating existing methods. However, assessment methods should be based on study goals. Level 1 strategies are effective at evaluating levels of insecticide resistance but may not reflect the performance of a formulated product in the field. Level 2 strategies directly measure product performance in the field against individuals of a specific mosquito population, but do not provide information about how the application impacted the field population. Level 3 strategies are designed to determine whether and how much a mosquito population was reduced following a mosquito control application, but do not provide supporting data about population susceptibility or application parameters. Ideally, a range of assessment strategies are used as an ensemble to ensure control activities are working as anticipated. Research is needed to develop standardized protocols and to continue to develop strategies for assessing mosquito control applications.

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OPS application: A tool for adapting to the modern mosquito control landscape in the Coachella Valley

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Introduction

The Coachella Valley Mosquito and Vector Control District (District) controls pathogen-carrying vectors in southeastern California to reduce the risk of disease transmission, such as the mosquito-borne West Nile virus. The district covers an area of 2,400 square miles, which includes marsh areas and residential communities that provide ample habitats for vectors, such as catch basins, drywells, swimming pools, agriculture runoff and duck clubs. Twenty-eight field technicians are responsible for site inspections, surveillance and preventative chemical applications. They also use biocontrol measures, such as releasing mosquito fish when necessary. Additionally, they conduct scheduled and call-requested inspections for controlling mosquitoes, red imported fire ants, eye gnats and flies. They also provide site inspections with advice on rodent-proofing properties and other nuisance and vector species. It is important for the District to efficiently

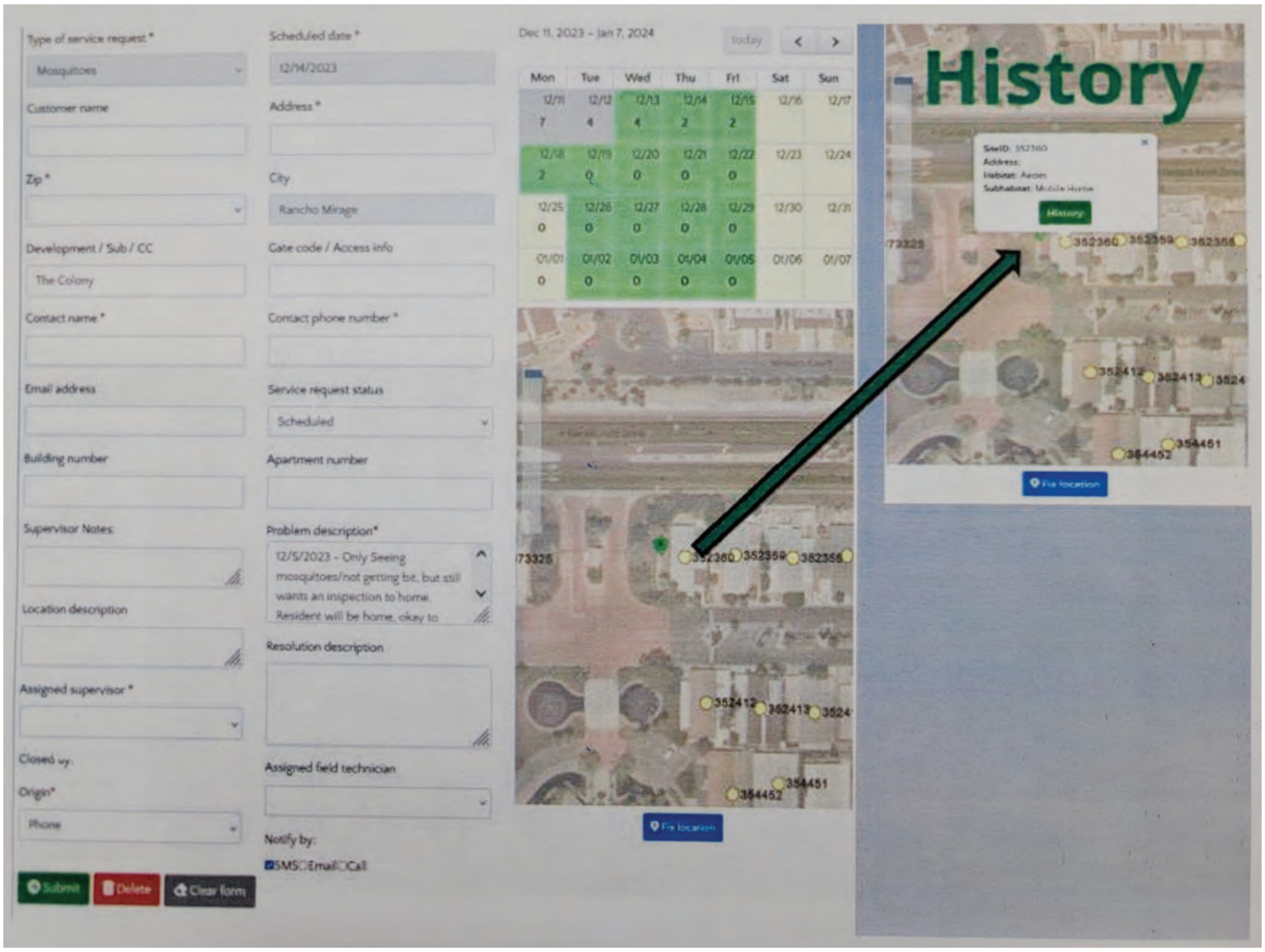

Figure 1.—Service Request form.

Figure 2.—Tech zone map displaying point and polygon mosquito sources.

perform vector control operations and field activities. The following overview describes the customized features of the operations "OPS" application that organizes day-to-day operations during arbovirus control efforts.

Methods

The OPS application is web-based, using the backend programming language C# and frontend languages HTML 5, Bootstrap and JavaScript. The application uses the

Figure 3.—Brood map displaying mosquito sources, based on selected control product residual expectancy.

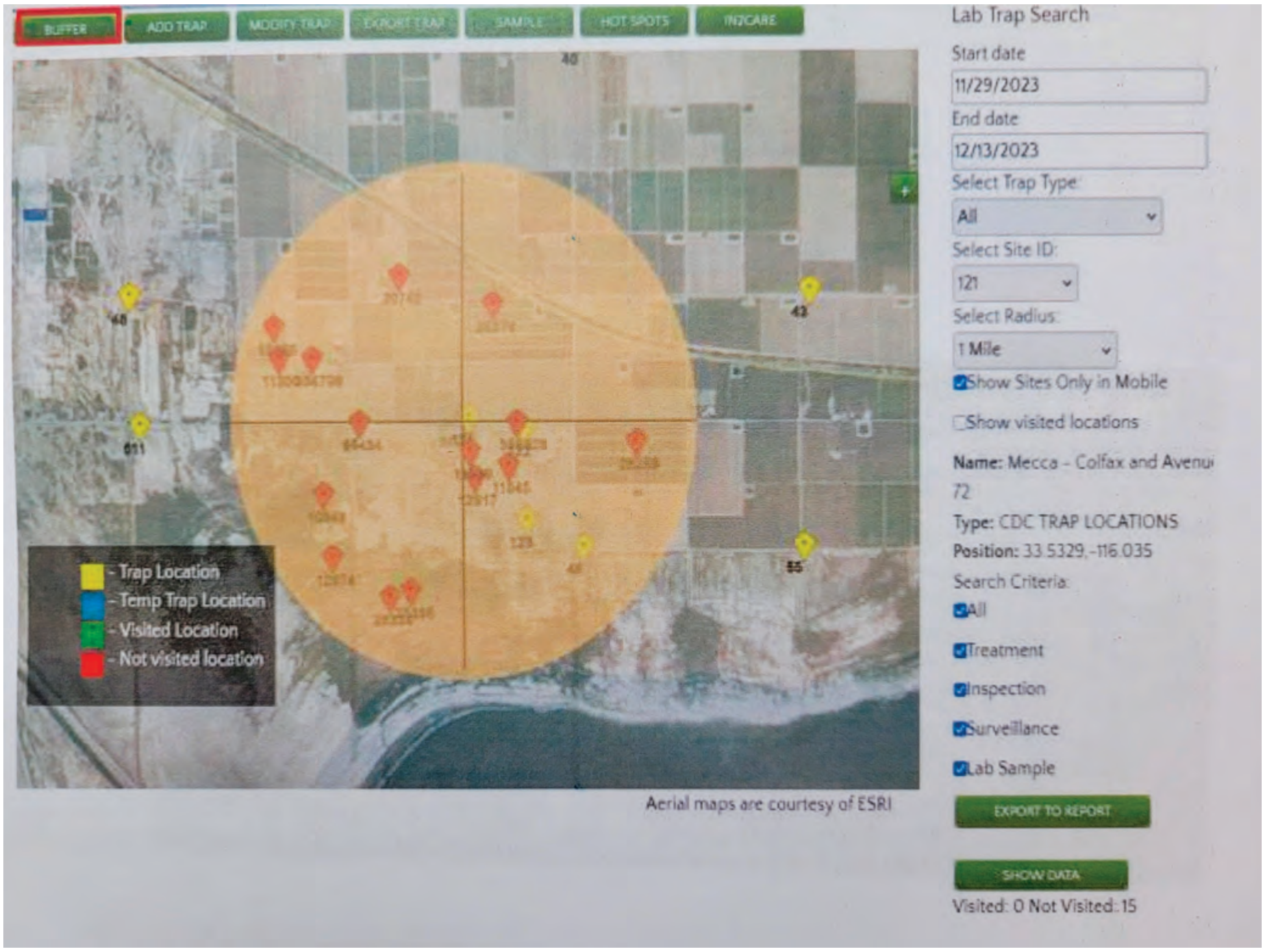

Figure 4.—Enhanced surveillance trap data map displaying trap locations ‘Yellow Marker’ and not visited locations ‘Red Marker’.

MSSQL 2022 and Redis databases. The OPS application has many custom features. Firstly, the ‘service request’ is a formal request for service from a resident. The service request form gets populated by the call center with type of service, schedule date, address, contact information, phone number, email, and problem description. Next, the supervisor assigns the service request to a vector control technician. The technician can see if there had been any previous inspections, treatments, or mosquito surveillance at this location. Then the technician reads the problem description to determine a course of action (Figure 1). Secondly, the ‘tech zone’ map displays point and polygon sources. Points are areas where mosquito sources are found, such as drywells, catch basins, and drains. Polygons are larger scale areas where mosquitos can be found such as agriculture, date palms, duck club ponds and marshes (Figure 2). Thirdly, a ‘brood’ map is an aerial map displaying treated mosquito areas which helps identify when to reinspect based on control product residual expectancy (Figure 3). Fourthly, an enhanced surveillance map is used to display locations of traps where mosquitos have tested positive for a virus. The yellow marker represents each trap location, while a red marker represents mosquito sources that have not been recently inspected. Once a trap has been identified as a positive, a one-mile perimeter buffer is established to determine mosquito sites that have not been inspected in the last two weeks. (Figure 4). Fifthly, the ‘work assigned’ map focuses attention on high mosquito production sites as shown as red with green points on the map (Figure 5). Lastly, OPS application is a centralized repository that produces valuable reports, such as neglected swimming pools per city in the Coachella Valley (Figure 6). OPS application reports and customized features are grouped into different categories that provide supervisors with important details that help to plan, budget, track progress, concentrate District efforts, and improve decision-making.

Results and Discussion

The OPS application provides a precise data collection system that enhances control technician productivity which, in turn, reduces mosquito populations and virus activity. For instance, the application delivers accurate calculations for acreage and chemical applications and enables technicians to optimize their workflow, minimize errors and record local information for future use. Technicians can

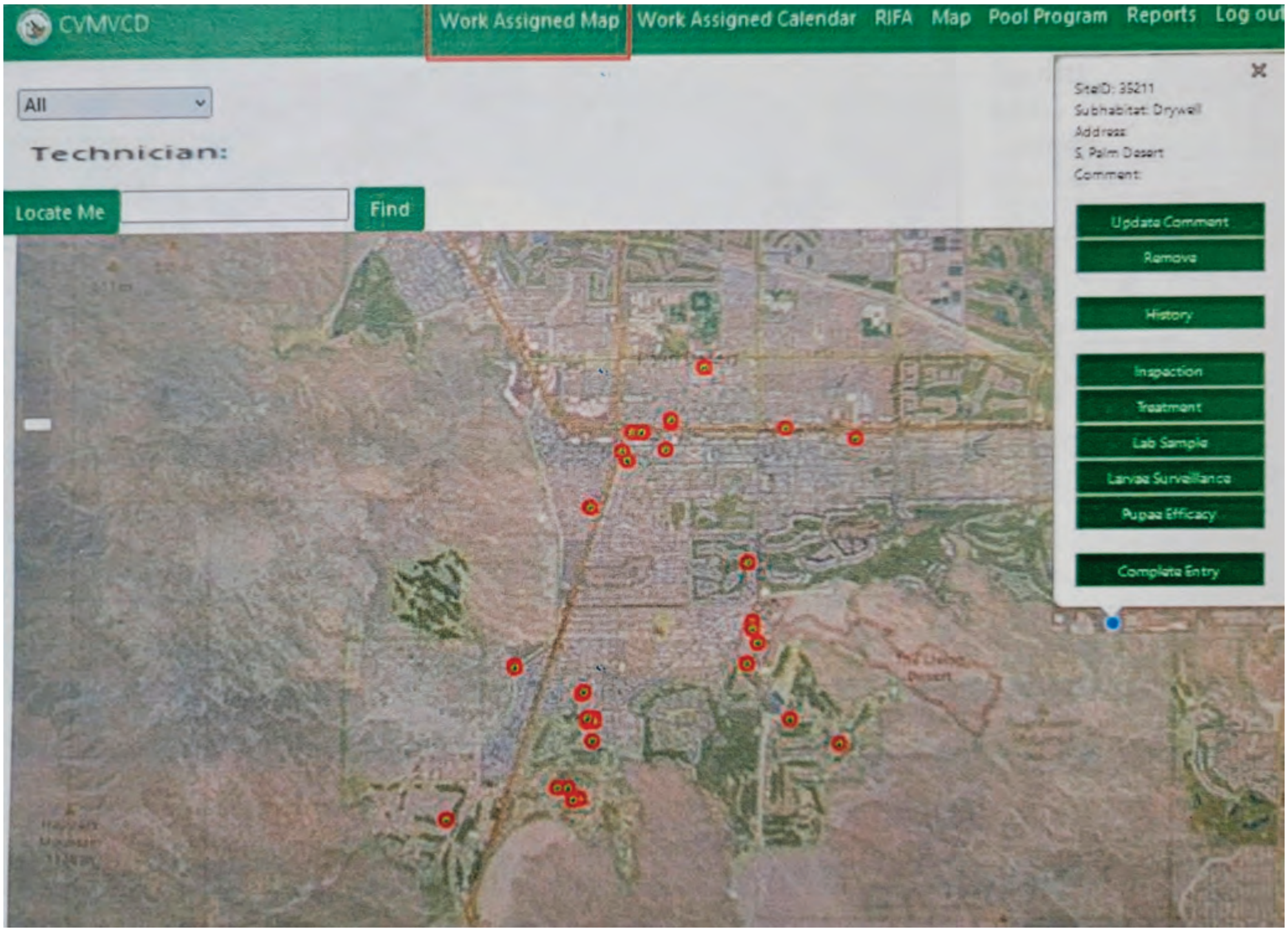

Figure 5.—Work assigned map focussing on high priority mosquito sources.

conveniently access aerial photos, maps, service requests, task-oriented forms and inspection site histories. The inspection site history provides valuable information about chemical usage and exact quantities, enabling users to make informed decisions based on past performance. Tech zones, brood, and enhanced surveillance maps aid in prioritizing sites or locations. The OPS application is an exceptional tool that facilitates supervisors to assign daily work using the scheduling vector location dashboard and to prepare daily, weekly or monthly reports within minutes, eliminating the requirement for support personnel to gather data that previously took several hours.

Conclusions

The District in-house OPS application is dynamic and adaptable to the ever-changing landscape of mosquito control in the Coachella Valley. Our solution has enabled us to consolidate scattered data into a single repository, significantly improving control operations and thereby reducing mosquito abundance. We anticipate that the OPS application will continue to evolve and adapt. Our next step involves the integration of AI for Vector Location Scheduling and routing, a move that will bolster the application's capabilities and further enhance our mosquito control efforts.

Acknowledgements

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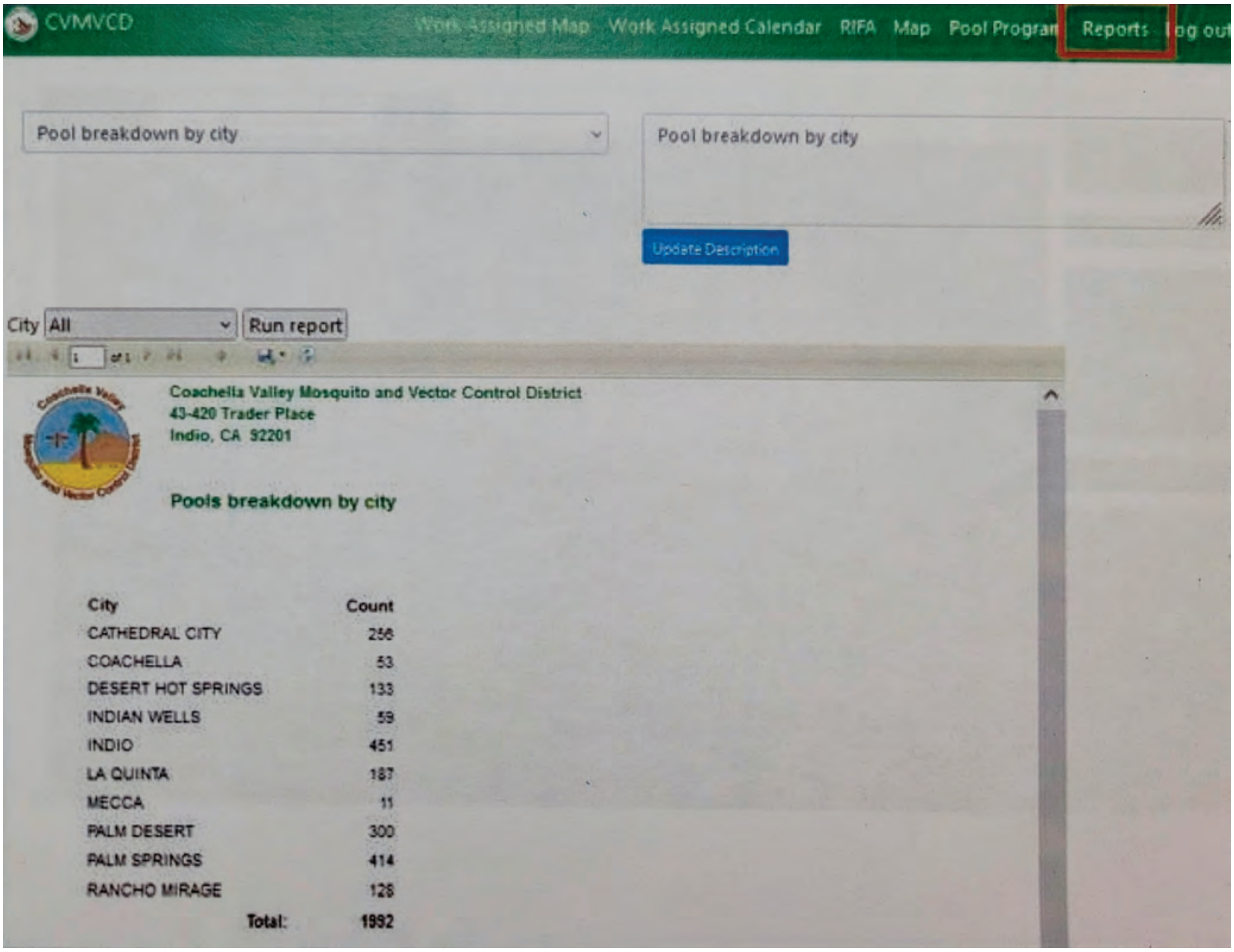

Figure 6.—Reports: Neglected swimming pool breakdown by city in the Coachella Valley.

Surveillance for mosquito-borne virus activity in California, 2023

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Abstract

In 2023, 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 43 of 58 counties in California, and St. Louis encephalitis virus (SLEV) activity was reported from 18 counties. A total of 473 human WNV infections were reported, and non-human WNV activity was detected among horses, dead birds, mosquitoes, and sentinel chickens. Nineteen human cases of SLEV disease were identified in ten counties, and enzootic SLEV activity was detected in mosquitoes from fifteen counties and in one sentinel chicken from one county.

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 2023, 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 43 (74%) of 58 counties in California (Table 1), while SLEV activity was reported from 18 (31%) 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 an SLEV IgM MAC-ELISA and plaque reduction neutralization tests (PRNT) at VRDL. Additional WNV infections were identified through screening performed by blood centers.

In 2023, a total of 433 symptomatic and 40 asymptomatic infections in blood donors with WNV were reported (Tables 1 and 3). Of the 433 symptomatic cases, 334 (77%) were classified as West Nile neuroinvasive disease (WNND) (e.g., encephalitis, meningitis, acute flaccid paralysis, or other neurologic dysfunction) and 99 (23%) were classified as non-neuroinvasive disease; 18 (4.1%) cases were fatal. Cases were residents of 34 counties, and 273 (63%) were male. In 2023, WNV disease incidence in California was 1.1 cases per 100,000 persons. Incidence was highest

Table 1.—West Nile virus activity in California by county, 2023. Humans include asymptomatic infections detected through blood bank and organ donor screening. NT = None tested.

County	Humans	Horses	Dead birds	Mosquito pools	Sentinel chickens
Alameda	2	0	80	18	4
Alpine	0	0	NT	NT	NT
Amador	1	0	NT	NT	NT
Butte	19	1	2	69	33
Calaveras	1	0	NT	NT	3
Colusa	1	0	2	2	2
Contra Costa	12	0	95	26	11
Del Norte	0	0	NT	NT	NT
El Dorado	3	0	4	NT	NT
Fresno	25	2	1	232	NT
Glenn	4	0	NT	1	NT
Humboldt	0	0	NT	NT	NT
Imperial	0	0	NT	3	NT
Inyo	0	0	NT	2	NT
Kern	19	2	0	107	NT
Kings	11	1	NT	70	NT
Lake	6	0	8	26	0
Lassen	0	0	NT	NT	NT
Los Angeles	68	0	44	580	30
Madera	9	1	1	207	NT
Marin	0	0	2	0	NT
Mariposa	0	0	NT	0	NT
Mendocino	0	0	0	NT	NT
Merced	9	2	0	43	22
Modoc	0	0	NT	NT	NT
Mono	0	0	NT	0	NT
Monterey	2	0	0	NT	NT
Napa	0	1	4	7	NT
Nevada	1	0	1	NT	2
Orange	7	1	2	218	NT
Placer	6	1	43	177	NT
Plumas	0	0	NT	NT	NT
Riverside	21	3	69	182	NT
Sacramento	60	2	196	342	7
San Benito	0	0	1	0	6
San Bernardino	30	2	11	158	NT
San Diego	0	0	14	1	NT
San Francisco	0	0	0	0	NT
San Joaquin	16	3	20	607	NT
San Luis Obispo	2	2	1	0	NT
San Mateo	4	1	15	0	0
Santa Barbara	0	0	0	0	NT
Santa Clara	3	1	117	18	NT
Santa Cruz	0	0	0	0	NT
Shasta	6	0	2	151	5
Sierra	0	0	NT	NT	NT
Siskiyou	0	1	NT	NT	NT
Solano	7	0	18	24	11
Sonoma	1	0	16	6	NT
Stanislaus	34	2	6	293	NT
Sutter	7	0	6	66	19
Tehama	0	0	NT	NT	4
Trinity	0	0	NT	NT	NT
Tulare	25	1	5	566	10
Tuolumne	0	0	NT	NT	NT
Ventura	1	0	4	0	0
Yolo	45	0	67	294	10
Yuba	5	1	0	26	7
State Totals	**473**	**31**	**857**	**4,522**	**186**

Table 2.—St. Louis encephalitis virus activity in California by county, 2023. NT = None tested.

County	Humans	Mosquito pools[1]	Sentinel chickens
Fresno	3	160	NT
Imperial	0	3	NT
Inyo	0	1	NT
Kern	2	75	NT
Kings	2	44	NT
Los Angeles	1	0	0
Madera	0	47	NT
Marin	1	0	NT
Merced	0	12	0
Napa	0	1	NT
Placer	0	1	NT
Riverside	1	118	NT
Sacramento	1	0	0
San Joaquin	0	9	NT
Shasta	0	3	1
Stanislaus	5	23	NT
Tulare	1	228	0
Yolo	2	3	0
State Totals	**19**	**728**	**1**

[1] Positive mosquito pools included *Culex quinquefasciatus* (387), *Cx. tarsalis* (276), *Cx. pipiens* (33), *Cx. stigmatosoma* (28), *Cx. erythrothorax* (1), *Aedes melanimon* (2), and *Ae. aegypti* (1).

(17.7 cases per 100,000 persons) in Yolo County, whereas Los Angeles County reported the most cases (62, 14% of total) (Figure 1, Table 3). The median age of those with WNND was 60 years (range, 13 to 94 years), and among cases with non-neuroinvasive disease the median age was 54 years (range, 26 to 80 years). The median age of the 18 WNV-associated fatalities was 71 years (range, 27 to 94 years). Dates of symptom onset ranged from April 26 to December 10, with the peak occurring during epidemiological week 32 (August 6th - August 12), when 54 (13%) symptomatic infections occurred.

Nineteen symptomatic cases of SLEV infection also were identified in 2023. Twelve (63%) cases presented with neuroinvasive disease and two fatalities were reported. Cases were residents of ten counties (Table 2) and 15 (79%) were male. The median age was 62 years (range, 21 to 82 years) and dates of symptom onset ranged from May 20 to October 29.

In addition to human cases of WNV and SLEV, two locally transmitted cases of dengue were identified in Los Angeles County in 2023. These marked the first locally acquired cases of dengue, which is caused by a virus transmitted by *Aedes* mosquitoes, detected in California.

Mosquito Surveillance

In 2023, mosquito testing was performed at DART and 13 local mosquito and vector control agencies. A total of 1,807,993 mosquitoes (63,208 pools) collected in 41 counties were tested by a triplex real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) for SLEV, WEEV, and WNV viral RNA (Table 4). *Aedes aegypti* mosquitoes also were tested for chikungunya,

Table 3.—Reported West Nile virus human cases by county of residence and year, California, 2014 – 2023.

County	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2023 incidence per 100,000 person-years	Ten-year incidence per 100,000 person-years
Alameda	1	0	0	1	0	1	0	0	1	1	0.07	0.03
Alpine	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Amador	0	0	1	0	1	1	0	0	0	0	0.00	0.75
Butte	24	53	21	4	12	5	4	13	3	18	8.76	7.64
Calaveras	0	0	0	0	0	0	0	0	0	1	2.23	0.22
Colusa	3	1	2	0	0	1	0	0	1	1	4.59	4.13
Contra Costa	5	1	4	4	4	1	4	2	1	10	0.87	0.31
Del Norte	0	0	0	0	0	0	0	0	0	0	0.00	0.00
El Dorado	0	0	1	0	0	0	1	1	0	3	1.59	0.32
Fresno	43	8	14	13	14	51	10	14	30	23	2.27	2.17
Glenn	10	19	6	0	2	0	1	2	1	4	13.97	15.71
Humboldt	0	0	0	0	1	0	0	0	0	0	0.00	0.07
Imperial	1	1	0	3	0	3	1	0	0	0	0.00	0.50
Inyo	0	0	0	4	0	0	0	0	0	0	0.00	2.12
Kern	11	11	17	30	13	28	8	8	22	16	1.76	1.81
Kings	4	0	8	5	0	3	2	8	7	9	5.96	3.05
Lake	1	2	1	0	1	0	2	0	0	6	8.98	1.95
Lassen	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Los Angeles	253	286	151	277	43	31	90	16	61	62	0.67	1.30
Madera	3	4	6	2	4	3	6	3	3	9	5.69	2.72
Marin	0	1	0	0	0	0	0	0	0	0	0.00	0.04
Mariposa	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Mendocino	1	2	0	0	0	0	0	0	0	0	0.00	0.34
Merced	1	1	0	10	2	10	12	6	7	8	2.80	2.00
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	0	0	1	0	1	0	0	0	0	2	0.46	0.09
Napa	0	0	0	0	1	0	0	0	0	0	0.00	0.07
Nevada	0	2	0	0	1	0	0	0	0	1	0.99	0.40
Orange	263	92	32	33	9	5	17	3	9	6	0.19	1.49
Placer	7	0	7	0	9	1	2	2	2	6	1.46	0.88
Plumas	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Riverside	14	127	11	32	15	12	10	3	0	20	0.82	1.00
Sacramento	10	4	25	6	15	4	7	6	5	53	3.37	0.86
San Benito	0	0	0	0	0	0	0	0	0	0	0.00	0.00
San Bernardino	21	54	8	57	9	7	3	1	4	28	1.28	0.87
San Diego	11	42	20	2	2	3	1	3	3	0	0.00	0.27
San Francisco	0	0	0	1	0	0	0	1	0	0	0.00	0.02
San Joaquin	9	2	13	14	14	7	2	7	4	15	1.91	1.11
San Luis Obispo	0	0	0	0	0	2	0	2	0	2	0.72	0.22
San Mateo	0	0	0	0	0	0	0	1	1	3	0.41	0.07
Santa Barbara	0	0	0	0	0	0	0	2	0	0	0.00	0.05
Santa Clara	10	8	1	0	1	1	0	3	1	3	0.16	0.14
Santa Cruz	0	0	0	0	0	0	0	1	0	0	0.00	0.04
Shasta	2	3	1	1	1	0	2	3	1	6	3.34	1.11
Sierra	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Siskiyou	0	1	0	0	0	0	0	0	0	0	0.00	0.23
Solano	5	1	4	1	0	1	1	2	2	6	1.35	0.52
Sonoma	0	0	0	0	0	0	0	0	0	1	0.21	0.02
Stanislaus	33	13	26	28	15	16	35	5	15	33	6.04	4.01
Sutter	8	2	12	3	1	1	1	0	1	7	7.07	3.64
Tehama	4	5	5	2	2	0	2	0	3	0	0.00	3.58
Trinity	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Tulare	21	13	10	12	8	24	7	8	15	25	5.26	3.01
Tuolumne	0	0	0	0	1	0	0	0	0	0	0.00	0.18
Ventura	1	6	7	1	2	2	0	0	0	1	0.12	0.24
Yolo	15	8	16	6	11	1	4	3	3	39	17.66	4.80
Yuba	6	10	11	1	2	0	0	0	1	5	6.05	4.35
Total WNV Cases	**801**	**783**	**442**	**553**	**217**	**225**	**235**	**129**	**207**	**433**	**1.11**	**1.03**
Asymptomatic Infections	91	77	41	47	26	18	28	19	14	40		
Total WNV infections	**892**	**860**	**483**	**600**	**243**	**243**	**263**	**148**	**221**	**473**		

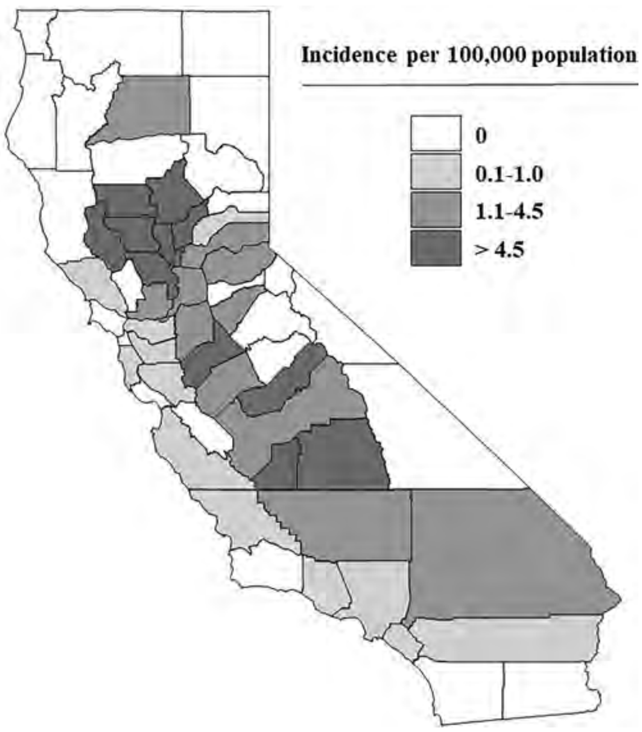

Figure 1.—Incidence of human cases of West Nile virus by county in California, 2023.

dengue, and Zika viruses at DART by a separate triplex RT-qPCR.

West Nile virus was detected in 4,522 mosquito pools from 31 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 2.5. During California's peak transmission period (July – September) the statewide MIR in *Culex* mosquitoes was 4.7 and 13 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, 2023). West Nile virus was detected from five *Culex* species (*Cx. erythrothorax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, and *Cx. tarsalis*), one *Aedes* species (*Ae. aegypti*), and one *Culiseta* species (*Cs. incidens*). (Table 5). WNV positive pools were collected from January 19 to December 19, with the peak occurring during epidemiological week 33 (August 13 – August 19).

SLEV was detected in 728 mosquito pools from 15 counties (Table 2). Statewide, the SLEV MIR in all mosquitoes tested was 0.4; the MIR was highest (6.7) in Kings County. St. Louis encephalitis virus was identified from five *Culex* species (*Cx. erythrothorax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, and *Cx. tarsalis*) and two *Aedes* species (*Ae. aegypti* and *Ae. melanimon*) collected from May 26 to October 31.

A total of 38,779 *Ae. aegypti* were additionally tested for chikungunya, dengue, and Zika viruses; all were negative.

Chicken Serosurveillance

In 2023, 24 local mosquito and vector control agencies in 20 counties maintained 77 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 3,790 chicken blood samples tested from 466 chickens, 186 (n = 466, 40%) seroconversions to WNV were detected among 54 flocks in 17 counties (Tables 1 and 4). Seroconversions to WNV occurred between July 14 and October 25, with the peak occurring during epidemiological week 34 (August 20 – August 26). One SLEV seroconversion was also detected from one county during epidemiological week 37.

Dead Bird Surveillance

In 2023, the WNV and Dead Bird Call Center and website received a total of 6,793 dead bird reports from 51 counties (Table 6). Tissue samples from dead bird carcasses or oral swabs transferred to RNA preservation cards (Fortius Bio, San Diego, CA) were tested at DART or at one of 13 local agencies by RT-qPCR. Of the 2,049 bird carcasses that were deemed suitable for testing, WNV was detected in 857 (42%) carcasses from 31 counties (Tables 1 and 6). Forty-eight different bird species tested positive for WNV: 60% were American crows, 13% were California scrub-jays, 5% were other corvids, and 22% were non-corvid or unknown species. Positive birds were detected from April 14 to December 31, with the peak occurring during epidemiological week 35 (August 27 – September 2).

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 2023, WNV infection was confirmed in 31 horses from 20 counties (Table 1). Eight (26%) of the horses died or were euthanized because of their infection.

Discussion

In 2023, 43 (74%) of 58 counties reported WNV activity. A total of 433 symptomatic human cases were reported from 34 counties, which was the highest number of cases reported in California since 2017 (Figure 3) and twice the 5-year average. Los Angeles County reported the most cases (n = 62), but the incidence was highest in Yolo County (Table 3). Non-human WNV activity was reported from 41 counties and all environmental indicators were higher than the previous year. Notably, the percentage of WNV positive dead birds was the highest reported since 2016, and the number of WNV positive mosquito pools was the highest ever reported in California. The percentage of WNV positive sentinel chicken flocks was also higher compared to

Table 4.—Results of mosquito and sentinel chicken testing for West Nile virus by county, California, 2023.

County	No. mosquitoes tested	No. mosquito pools tested	WNV + pools	No. flocks	No. chickens	No. WNV positive flocks	WNV + sera
Alameda	23,152	896	18	3	20	3	4
Butte	21,706	488	69	7	45	7	33
Calaveras				1	10	1	3
Colusa	500	10	2	1	10	1	2
Contra Costa	21,648	732	26	4	22	3	11
Fresno	73,883	2,139	232				
Glenn	794	16	1				
Imperial	2,824	215	3				
Inyo	3,949	81	2				
Kern	64,348	1,635	107				
Kings	22,814	484	70				
Lake	13,769	447	26	2	12	0	0
Los Angeles	187,535	4,587	580	21	81	13	30
Madera	22,974	643	207				
Marin	2,548	160					
Mariposa	213	8					
Merced	27,761	1,007	43	8	48	6	22
Mono	300	6					
Napa	10,062	309	7				
Nevada				2	12	1	2
Orange	162,340	5,398	218				
Placer	30,086	1,823	177				
Riverside	293,680	8,325	182				
Sacramento	69,423	5,144	342	3	17	2	7
San Benito	74	24		1	8	1	6
San Bernardino	57,553	2,934	158				
San Diego	20,415	2,311	1				
San Francisco	95	8					
San Joaquin	128,385	3,066	607				
San Luis Obispo	543	15					
San Mateo	7,175	497		2	13	0	0
Santa Barbara	3,287	143					
Santa Clara	24,153	3,414	18				
Santa Cruz	1,312	91					
Shasta	73,996	2,214	151	3	20	1	5
Solano	15,660	399	24	3	20	3	11
Sonoma	11,456	528	6				
Stanislaus	89,626	2,235	293				
Sutter	14,464	374	66	5	32	5	19
Tehama				3	27	2	4
Tulare	237,143	7,339	566	1	10	1	10
Ventura	953	24	0	3	30	0	0
Yolo	57,947	2,842	294	2	17	2	10
Yuba	7,447	197	26	2	12	2	7
Total	**1,807,993**	**63,208**	**4,522**	**77**	**466**	**54**	**186**

previous years (Figures 4, 5 and 6; California Department of Public Health). Additionally, the number of WNV equine cases was the highest observed since 2008. 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.

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. This resulted in the identification of 19 human SLEV cases from ten counties; the highest number of SLEV cases reported since the re-emergence of this virus. A total of 728 SLEV positive mosquito pools were reported from 15 counties, including six of the counties with reported SLEV cases (Table 2), which is the highest number of SLEV positive mosquito pools reported in California since it reemerged. Inyo and Napa counties reported SLEV positive mosquito pools for the first time since the statewide arbovirus surveillance program began in 1969. Notably, Shasta County reported an SLEV seroconversion in one chicken concurrent to an SLEV positive mosquito pool detection and marked the first time that virus had been detected in that county

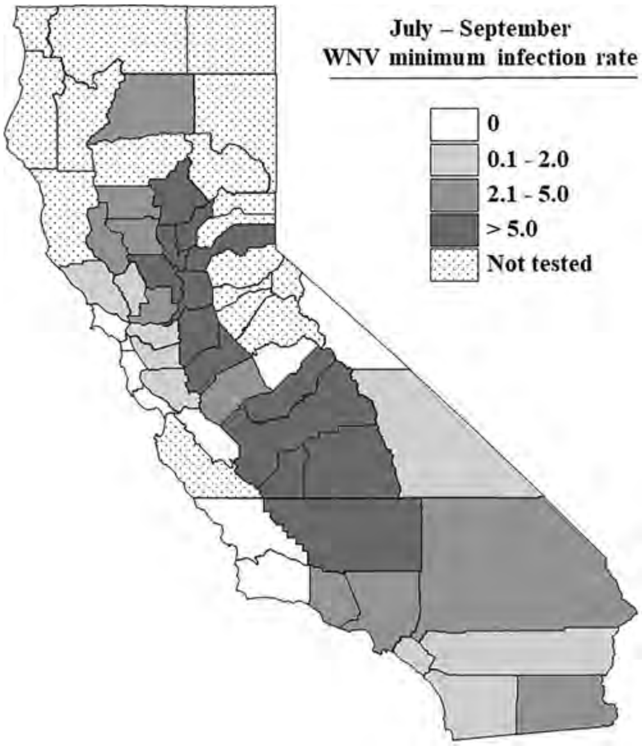

Figure 2.—West Nile virus minimum infection rate in *Culex* mosquitoes, by county, California, July – September, 2023. Minimum infection rate is defined as the minimum number of infected female mosquitoes per 1,000 tested.

Table 5.—Mosquito species tested for West Nile virus, California, 2023.

	No. pools	No. mosquitoes	WNV+	MIR
Culex species				
Cx. erythrothorax	2,273	85,444	9	0.1
Cx. pipiens	11,046	199,605	705	3.5
Cx. quinquefasciatus	22,478	690,367	1,797	2.6
Cx. restuans	17	40	0	0.0
Cx. stigmatosoma	796	9,644	51	5.3
Cx. tarsalis	25,552	809,820	1,956	2.4
Cx. thriambus	38	50	0	0.0
Culex species	14	262	1	3.8
All *Culex*	**62,214**	**1,795,232**	**4,519**	**2.5**
Anopheles species				
An. franciscanus	1	1	0	0.0
An. freeborni	6	109	0	0.0
An. hermsi	1	10	0	0.0
All *Anopheles*	**8**	**120**	**0**	**0.0**
Aedes species				
Ae. aegypti	595	6,246	2	0.3
Ae. melanimon	28	987	0	0.0
Ae. nigromaculis	2	22	0	0.0
Ae. vexans	1	2	0	0.0
All *Aedes*	**626**	**7,257**	**2**	**0.3**
Other species				
Culiseta incidens	252	3,801	1	0.3
Cs. inornata	102	1,379	0	0.0
Psorophora columbiae	1	3	0	0.0
Unknown	5	201	0	0.0
All other	**360**	**5,384**	**1**	**0.2**

Table 6.—Dead birds reported, tested, and positive for West Nile virus, California, 2023.

County	Reported	Tested	Positive	Percent
Alameda	505	158	80	51
Alpine	0			
Amador	2			
Butte	72	18	2	11
Calaveras	5			
Colusa	10	4	2	50
Contra Costa	556	131	95	73
Del Norte	0			
El Dorado	59	17	4	24
Fresno	138	10	1	10
Glenn	3			
Humboldt	10			
Imperial	2			
Inyo	1			
Kern	16	2		
Kings	14			
Lake	25	16	8	50
Lassen	0			
Los Angeles	812	128	44	34
Madera	29	12	1	8
Marin	50	4	2	50
Mariposa	3			
Mendocino	18	2		
Merced	41	2		
Modoc	0			
Mono	4			
Monterey	16	1		
Napa	29	10	4	40
Nevada	17	6	1	17
Orange	169	43	2	5
Placer	276	146	43	29
Plumas	0			
Riverside	265	113	69	61
Sacramento	941	456	196	43
San Benito	8	2	1	50
San Bernardino	119	25	11	44
San Diego	213	96	14	15
San Francisco	68	15		
San Joaquin	152	43	20	47
San Luis Obispo	34	12	1	8
San Mateo	348	84	15	18
Santa Barbara	16	10		
Santa Clara	642	199	117	59
Santa Cruz	44	12		
Shasta	25	6	2	33
Sierra	0			
Siskiyou	1			
Solano	123	42	18	43
Sonoma	177	25	16	64
Stanislaus	165	15	6	40
Sutter	51	11	6	55
Tehama	7			
Trinity	0			
Tulare	52	13	5	38
Tuolumne	3			
Ventura	91	25	4	16
Yolo	340	132	67	51
Yuba	26	3		
Totals	**6,793**	**2,049**	**857**	**42**

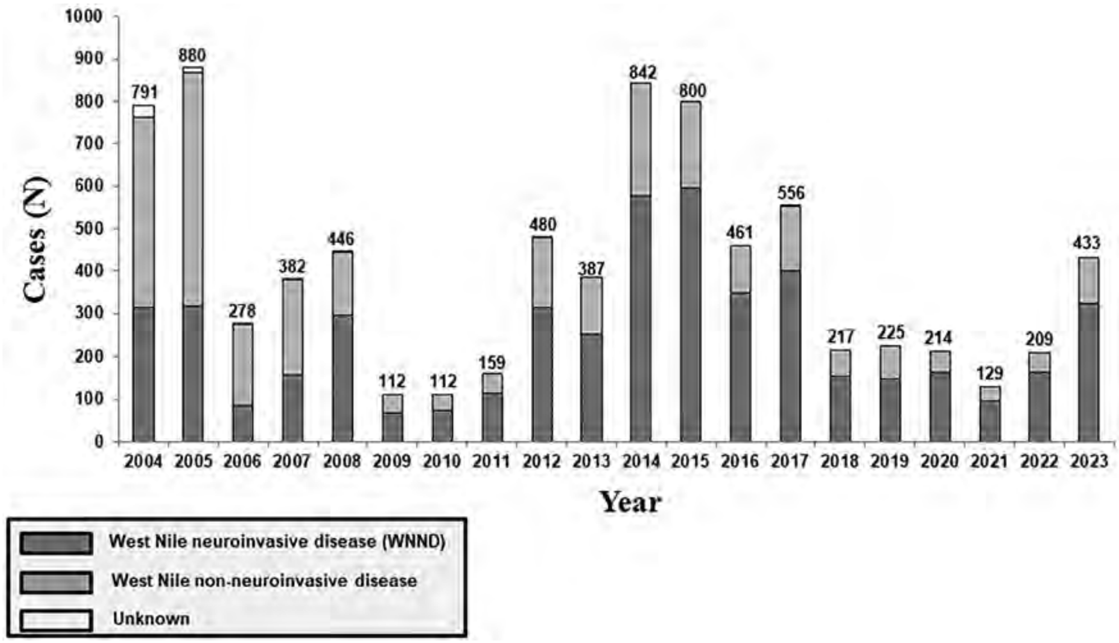

Figure 3.—Human cases of West Nile virus in California, by year, 2004 – 2023.

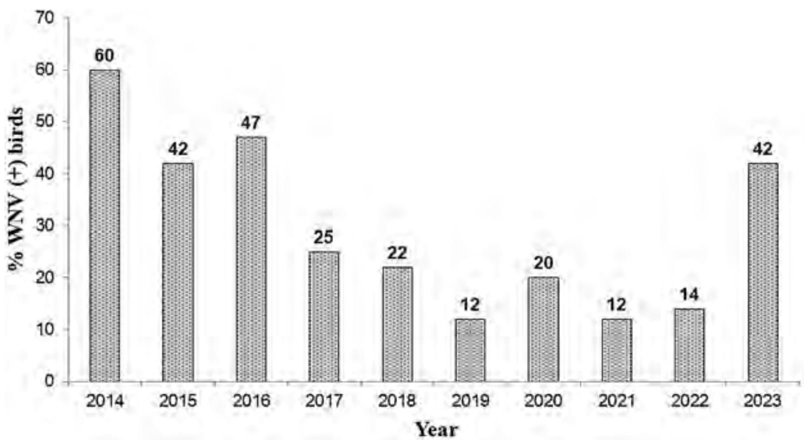

Figure 4.—Percentage of dead birds positive for West Nile virus in California, 2014 – 2023.

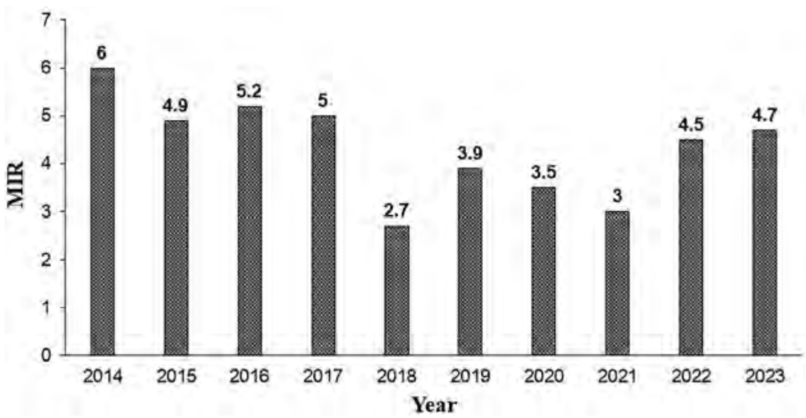

Figure 5.—Minimum infection rate (MIR) in *Culex* females per 1,000 tested for West Nile virus in California, July – September, 2014–2023.

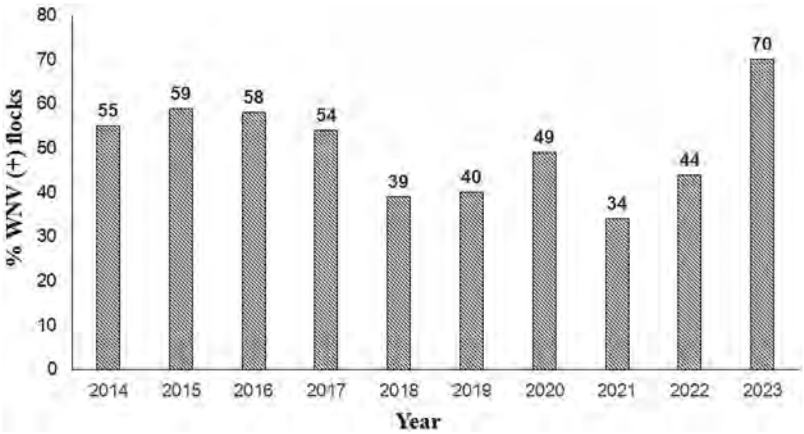

Figure 6.—Percentage of sentinel chicken flocks in California with one or more birds positive for antibodies to West Nile virus, 2014–2023.

since 1972 (Table 2). The expansion of SLEV to these three additional counties highlights the importance of using multiple environmental surveillance tools to detect arbovirus activity.

Notably, the first ever locally acquired human cases of dengue were reported in California this year from Los Angeles County. However, follow-up testing of *Ae. aegypti* and *Ae. albopictus* mosquitoes did not detect any dengue positive mosquito pools. Despite this, these mosquito species continued to be routinely tested for chikungunya, dengue, and zika viruses throughout the state to maintain vigilance for the introduction of these exotic arboviruses into California. 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.

Conclusions

Environmental activity of WNV was higher in 2023 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 disease were highest since 2017 and SLEV human disease cases were the highest reported since the re-emergence of SLEV in CA, highlighting the importance for ongoing surveillance and awareness of potential human disease risk. Environmental detections of both viruses often preceded the 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 Christina De Cesaris, Sandra Garcia, Kurt Johnson, Aynaz Lotfata, Kylie Pace, Shawn Ranck, Lincoln Wells, and Olivia Winokur), the California Animal Health and Food Safety Laboratory, and the CDFA Animal Health Branch. From CDPH, we thank VRDL (especially Cynthia Bernas, Brandon Brown, Teal Bullick, Lyndsey Chaille, Nick D'Angelo, Mojgan Deldari, Bianca Gonzaga, Ydelita Gonzales, Kim Hansard, Carl Hanson, Deidra Lemoine, Maria Liu, Ruth Lopez, Leo Oceguera, Nichole Osugi, Peter Patiris, Chris Preas, Clarence Reyes, Maria Salas, Maria Uribe-Fuentes, and Shigeo Yagi), the Veterinary Public Health Section (especially Curtis Fritz), the Infectious Diseases Branch (especially Allyx Nicolici), and VBDS (especially Ervic Aquino, Robin Hinks, Margaret Kerrigan, Amanda Niemela, Mary Joyce Pakingan, and Erin Trent.).

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A day in the life of a Service Request

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Abstract

Our presentation describes how a customer service request or complaint is handled operationally by various organizations using ArcGIS Online and FieldSeeker GIS. When service requests are managed in GIS, there are many options for collection, management, routing, assignment, notification, and automatic closing. We present a few examples using Web GIS.

Targeting: Directing Surveillance Efforts to Optimally Deploy Control Resources

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Abstract

West Nile Virus (WNV) is considered an endemic arbovirus across the state of California, with human transmission evident since 2003. In the proceeding years, vector and mosquito abatement services have tailored aspects of their programs to mitigate and respond to the increasing prevalence of WNV transmission with the aim of preventing human disease. At the San Gabriel Valley Mosquito and Vector Control District, a modified surveillance methodology, referred to as Targeting, has been adopted to assess and prioritize locations of known or increased WNV prevalence. Targeting involves selecting a site in need of investigation, delineating a half mile radius area surrounding the site, dividing the selected area into five quadrants and identifying potential trap locations within each of the five quadrants. The central quadrant targets a 150-meter radius area around the initial investigation site, whereas the other four quadrants target the directional corners of the total selected area. Depending on the type of investigation, different trap types are utilized for surveillance. For *Culex* species or WNV investigations Reiter-Cummings Gravid traps were utilized and for *Aedes* species or when dengue/chikungunya/Zika cases were under investigation Biogents Sentinel 2 traps were utilized. One trap is deployed per delineated quadrant. By designating a specific area of surveillance and repeating the methodology several times in different WNV targeted areas, an incident vector index can be calculated for each event to rank the relative threat from each targeted area. This provided direct operational objectives based on the calculated vector index. The strength of the process was the standardization of several response triggers into one process to gauge the relative risk within specific areas and applying that standard to a wide variety of scenarios. This standardization allowed for an expedited and predictive response that could be prepared for in the latter months of the mosquito season. The San Gabriel Valley MVCD plans to continue using this methodology as a tool to gauge the relative risk that WNV transmission poses to its local human residents.

Incorporating Sterile Insect Technique into IPM toolbox to control invasive *Aedes* mosquitoes in the West Valley region of San Bernardino County, California

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Abstract

Sterile Insect technique (SIT) has brought opportunities to intensify efforts to control invasive *Aedes* in California. The West Valley Mosquito and Vector Control District has embraced this tool and is working to incorporate it into the conventional IPM toolbox for invasive *Aedes* mosquito control. Our approach is unique because it only targets *Aedes* hotspots instead of area-wide mass releases. This work aims to assess the impact of targeted X-ray sterilized male mosquito releases on the local population dynamics of invasive *Aedes* mosquitoes. In our pilot program, we released X-ray sterilized male *Ae. aegypti* at three locations in the West Valley region of San Bernardino County. First, a site was selected based on counts from weekly surveillance data using BG Sentinel traps. Baseline (prior to release) and follow-up cluster mosquito trapping was conducted within 100 and 200 yards from each site. Irradiated male mosquitoes, a 100-times the number of female *Ae. aegypti* from BG Sentinel traps, were released at each site. Follow-up cluster mosquito trapping was conducted at 9 sites around each release site for eight consecutive weeks. The results indicated a reduction of *Ae. aegypti* population by as high as 71% four weeks after release. Preseason sterile mosquito releases are currently underway. Lessons learnt from this pilot program will help to optimize SIT as an additional tool in our invasive mosquito control program.

Preparing the public for sterile mosquito releases

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Abstract

Given the ever-expanding range of the invasive *Aedes aegypti* mosquito, the methods to combat this cryptic-breeding, aggressive day-biting mosquitoes need to expand as well. The West Valley Mosquito and Vector Control District (District) decided that X-Ray irradiation of *Ae. aegypti* males would best serve its residents. We knew it would take a clear, concise, science-based approach to prepare the general public for the release of additional mosquitoes, given they are already suffering from the additional biting pressure and the rampant conspiracy theories on the internet concerning Bill Gates as well as GMO mosquitoes.

The two-year process was built upon the California Special District Association (CSDA) District Transparency Certificate of Excellence and the award from the California Department of Pesticide Regulation as an Integrated Pest Management (IPM) innovator in an effort to build trust. From there the District p[roduced a full year of IPM messaging stressing both physical and biological control, the appropriate category for Sterile Insect Technique (SIT). IPM positive messaging included highlights during both Mosquito Awareness Week and Mosquito Control Awareness Week. The development and design of these graphics was coordinated with a professional graphic designer to ensure continuity of design and messaging.

The District then focused on the development of SIT FAQs and messaging for both staff and the public, presented as a part of the biological control aspect of IPM. Fortunately, the California Department of Food and Agriculture was also publicizing the control and release of sterile male Mediterranean Fruit Flies within District boundaries, a practice that has been ongoing since the 1980s. Because both insects are sterilized using X-Ray irradiation, a technology familiar to the general public, we were able to tie some of the outreach concepts together.

By building a comprehensive strategy for outreach of IPM and SIT releases, the District has encountered little to no resistance from the community.

The Southern California SIT Joint Pilot Project update year Two: The journey of developing engagement, strategies, and processes for X-ray sterilized male *Aedes aegypti* releases

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Abstract

The partnership between the Orange County Mosquito and Vector Control District (OCMVCD) and the Greater Los Angeles County Vector Control District (GLACVCD) to conduct a multi-year joint sterile insect technique (SIT) project targeting invasive *Aedes aegypti* populations saw many important strides in its second year. During year two, the SIT Joint Project Team continued baseline trapping at planned release and control sites, enhanced mass rearing capabilities, innovated equipment, conducted nearly a dozen studies including tests of mosquito fitness, longevity, mating, chilling, compaction, dusting, and irradiation dosing. Additionally, both groups performed single-point and multi-point mark release recapture studies in their respective jurisdictions. The second year of the SIT Joint Project also featured aspects of community engagement involving meetings with trustees, city officials, HOAs, website and media materials development, and conducting door-to-door KAP surveys in both of the planned release residential neighborhoods. This presentation gave an overview of this work highlighting the challenges and the successes of a year filled with learning, growth, and collaboration.

Moving towards sterile male mosquito mass releases: Fitness assessments of *Aedes aegypti* (Diptera: Culicidae)

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Introduction

Invasive *Aedes aegypti* (L.) has been expanding its distribution throughout California and increasing in abundance for more than a decade (CDPH 2023, Metzger 2017). The sterile insect technique (SIT) is a potentially effective method for mosquito control to suppress population density (Dobson 2021, Gato et al. 2021). A SIT Joint Pilot Project partnership between the Orange County Mosquito and Vector Control District (OCMVCD) and the Greater Los Angeles County Vector Control District (GLACVCD) was developed and aimed at mass producing sterilized male *Ae. aegypti* using X-ray irradiation. The goal of the Project is to release sterilized males into study areas where they can compete with wild males for mating opportunities with wild females, resulting in non-viable eggs and ultimately population suppression. For this project, in-house chilling, compaction, irradiation, and dusting methods are essential steps for producing sterile male *Ae. aegypti* (Tussey et al. 2023). Although some of these procedures are still being developed, it is critical to assess the impact of the treatments individually and combined on the fitness of the release strain to ensure the production of a sterile but sufficiently competitive male for release.

Methods

A combination strain (Combo) of *Ae. aegypti* was developed by crossbreeding Mission Viejo (MV) Orange County, California and Greater Los Angeles, Los Angeles County, California (LA) strains and was utilized along with the MV strain for comparison in fitness studies. The Combo strain was produced by a third party, MosquitoMate®, in Kentucky and eggs were regularly shipped to OCMVCD for rearing to the adult stage, whereas the MV strain was housed and reared at OCMVCD. The survivorship and wing-lengths of MV and Combo males were assessed using a life table study, under semi-field microcosms in two different settings: a 50-car metal parking garage with two large bay doors at each end left open for 11 hours for five days of the week (barn), and outdoors shaded by vegetation. The effects of adult mosquito exposure to chilling and a sequential experiment of chilling and compaction were further examined. Fifty males per cage of both strains, fed with 10% glucose solution, were used for the life table studies, with three replicates for each treatment. The chilling condition was 4 °C for 10 min, and compaction was conducted at 4 °C for 15 min at a density of 100 mosquitoes per milliliter (1cm^3). Fitness was assessed in BugDorms 1ft^3 (W30 × D30 × H30 cm) (MegaView Science Co., Ltd., Taichung Taiwan) for the semi-field study, whereas larval-adult mosquito rearing chambers (Bioquip) were used for the groups with treatments. Kaplan–Meier survival analysis was used to determine the difference between strains and microcosms, and the effects of chilling or compaction treatments on the survivorship for each strain. Log-rank test was used to determine their statistical significance. All statistical analyses were performed using R version 4.2.3.

Results and Discussion

Without treatment, the MV strain survived significantly longer than the Combo strain over a 30-day period, inside the barn (Log-Rank test, $P = 0.0004$) and under vegetation ($P < 0.0004$). Similar survivorship results were found when only considering the first two weeks (14 days). These results determined that over 65% of the mosquitoes survived during the 30-day observation period in both settings for both *Ae. aegypti* strains. Literature suggests that mosquito wing length is positively correlated with fitness (Jeffrey Gutiérrez et al. 2020). We found that MV strain males had slightly longer wings (2.204 mm, 95% CI: 2.190, 2.219 mm) compared to the Combo strain (2.177 mm, 95% CI: 2.165, 2.189mm) (*t*-test, $P = 0.004$).

The application of chilling alone decreased the survivorship of MV train, but did not significantly influence survivorship, whereas the combination of chilling and compaction led to a significant decrease in survivorship for both strains. For the Combo strain, chilling for 10, 30 or 60 min did not affect the survivorship in comparison to controls with no chilling procedure (Log-Rank test, $P = 0.26$). For MV strain, chilling decreased the survivorship ($P < 0.0001$), but chilling time had no significant effect ($P = 0.40$). Overall, after being chilled, there was no survival difference between the two strains within 30 days ($P = 0.88$). However, the Combo survived better during the first 14 days compared to MV strain (survival rates: 75% vs. 66%, $P = 0.037$). When treated with chilling followed by compaction, the Combo strain survived better than the MV strain through the 30 experimental days (Log-Rank test, $P = 0.0017$). The median longevity was 14 days for the MV strain, and 17 days for the Combo strain.

Conclusions

Under semi-field conditions with no treatments, MV survived longer and had longer wing length than the Combo strain. The longer wing growth may have been due to differences in rearing conditions which were not held constant between strains. With chilling, there was no difference in longevity between the two strains. However, with chilling followed by compaction treatment, the Combo strain exhibited an excellent survivorship compared to untreated controls, while the MV strain showed reduced survival.

Based on the current fitness studies, both strains are potentially useful for mass release, as they demonstrated excellent survival under cold conditions and compaction, two critical steps in confining adult mosquitoes for irradiation treatment. We will continue to evaluate the combined effect of chilling, compaction, irradiation and dusting on male longevity, mating success, fertility and fecundity.

Acknowledgements

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Enhancing local preparedness: Mobilizing volunteer resources against local transmission of *Aedes*-borne disease in Los Angeles County

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Abstract

The introduction and subsequent establishment of *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.) in Los Angeles County have created many operational control and surveillance challenges for local vector control agencies. The rapid increase in distribution and abundance of these species have elevated the risk for autochthonous transmission of viruses such as dengue, chikungunya and Zika, which are regularly detected as travel-associated human infections by the Los Angeles County Department of Public Health (LACDPH). Conducting targeted surveillance, control, and outreach efforts around these human cases to mitigate the risk of local transmission is highly resource-intensive, particularly when concurrently responding to two or more probable or confirmed cases. Consequently, in the event of an outbreak of *Aedes*-transmitted disease, vector control agencies can become easily overwhelmed. In 2016, the Greater Los Angeles County Vector Control District (GLACVCD) partnered with the Emergency Preparedness Response Division of the LACDPH to facilitate access to public health volunteer resources during an outbreak or emergency declaration. Through this partnership, GLACVCD can access support from various governmental and community partners, including the Medical Reserve Corps, local Community Emergency Response Teams, and the Public Health Emergency Volunteer Network, to assist with conducting inspections and outreach efforts. Informational training sessions and full-scale exercises are conducted annually to ensure readiness. In this presentation, the evolution and current status of this partnership was discussed.

CalSurv development: New tools for vector control

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Introduction

CalSurv, a member of the VectorSurv family of applications, helps more than 60 mosquito and vector control agencies across California manage their data, generate reports, and make data-based decisions using integrated data analysis and visualization tools (VectorSurv Development Team 2024). The VectorSurv team develops and maintains CalSurv along with other VectorSurv applications, including the VectorSurv API and VectorSurv maps. Thanks to annual support from the state of California, the VectorSurv team continues to develop new tools for data management, visualization, and integration with other software tools. This presentation will review recent developments, discuss what the future holds for CalSurv, and explain more about the new VectorSurv application programming interface (API) which is the future of CalSurv data integration.

Methods

The CalSurv team identifies development projects by working with a variety of stakeholders including users of the CalSurv system in state and local public health agencies, the CalSurv Steering Committee, and 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. Recent and current development includes addressing the need for CalSurv data exchange using the VectorSurv API, additional functionality for tick surveillance, and modernization of the CalSurv gateway web interface. Future development is aimed at providing agencies with tools to manage their service visits, making the VectorSurv maps easier to use, and modernizing the calculator interface.

Results and Discussion

To better align the CalSurv tick functionality with the needs of vector control agencies within California, the VectorSurv team worked with the MVCAC Vector and Vector-Borne Disease Committee to identify features to enhance tick surveillance capability in CalSurv. To address these needs, CalSurv now allows additional vertebrate host options and new fields to allow agencies to record the following additional collection conditions: moisture, exposure to sunlight, and vegetation. The VectorSurv team is also developing a map showing a visual representation of tick surveillance. The map is being reviewed as part of the final stages of development and will be released to CalSurv users in 2025.

The VectorSurv team strives to provide CalSurv users with access to their data in a modern, standardized interface so they can view or manage their data using whatever tools best fit their workflow. The VectorSurv API is a RESTful API that allows for the integration of CalSurv data into other software applications using a framework already used by many software developers. Using the VectorSurv API, agencies or other software vendors can easily manage or view CalSurv data.

In CalSurv, users indicate the location of their traps by creating a site. The site is a representation of where one or more traps are located for a specified date range. The forms for defining sites are being updated as part of the modernization of the CalSurv interface. The new version of the site form has been recently developed and is being tested by the VectorSurv team in preparation for an upcoming release. This version follows modern software development design principles and is responsive to a wider variety of devices and screen sizes, including desktop and laptop computers, tablets, and smartphones. The new interface improves upon the functionality for making revisions to the site location over time. When a user moves a site, they create a revision indicating a new date range for the revision, and the location for the site within that date range. The new site revision interface provides more intuitive tools for viewing the history of revisions for a site and managing each site's revisions.

Following the development on the site forms, the next step in modernizing the CalSurv interface will be the mosquito collection and pool forms. The VectorSurv team plans to complete the new, modern version of the forms so that they are available by mid-April when usage increases by the majority of CalSurv agencies at the beginning of the mosquito surveillance season. Like with the site forms, the new interface will be easier to use on a variety of screen sizes. In early May, shortly after the release of the collection and pool forms, the VectorSurv team will provide online training for all CalSurv users and will make videos of the training session available online for future viewing.

CalSurv users have indicated a desire to record their service visits. CalSurv will soon have functionality to manage these records. An agency will be able to record a visit and associate related activity, such as data on any inspections completed, traps operated, or on any pesticide applications performed. A user will then be able to look up a location and view a history of activities at that location.

VectorSurv maps are a useful tool to visualize surveillance data. In 2024, the VectorSurv team will make some minor changes to the map interface to make it simpler to configure the desired view. In addition, the VectorSurv team will be making some changes to provide agencies with more control when embedding maps on their websites.

CalSurv provides calculators to analyze surveillance data to make data-based decisions. The VectorSurv team has identified several projects to enhance these calculators. The calculator interface will be modernized to use the same modern interface being developed for sites, mosquito collections, and mosquito pools. In addition, it will be possible to save calculator settings to make it easier to repeat commonly used calculations.

Acknowledgements

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Making use of your data: Decision making and reporting using CalSurv

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Abstract

CalSurv is data management, analysis, and reporting software powered by VectorSurv that is used widely across California to enable users to make real-time data-driven control decisions and customized reports. VectorSurv's built-in tools incorporate GIS capability to allow users to calculate abundance anomalies, pool virus infection rates, vector index, West Nile virus risk, and insecticide resistance over user-defined spatial regions. Recently, VectorSurv released the VectorSurv API, enabling CalSurv users to access their data programmatically within popular software tools such as Tableau, Power BI, Python, and R through widely used protocols. In 2023, VectorSurv released the VectorSurv API and increased the frequency of training and user support capacity to help agencies make better use of their data. All training sessions were recorded and are available on YouTube (youtube.com/@vectorsurv). Additionally, in early 2024, VectorSurv developed an R package, vectorsurvR (De Cesaris 2024), which allows for a more streamlined connection and workflow in R. Training plans for 2024 will be announced to all users via the VectorSurv newsletter (https://vectorsurv.org/newsletter).

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Evaluation of the sensitivity and utility of dead bird surveillance for West Nile virus in Sacramento and Yolo Counties

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Introduction

At the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD), a dead bird surveillance program plays an important role in the early detection of West Nile virus (WNV) activity. Therefore, continuous effort is made to improve the program. This has included expanding the types of samples collected from dead birds based on condition of the carcass and species of the bird. The detection of WNV-positive dead birds triggers increased mosquito WNV surveillance and larval mosquito control within a 0.25 to1.0-mile radius of the WNV-positive dead bird. Dead birds are reliable indicators of WNV activity (Nielsen and Reisen, 2007; Foss et al., 2015) and therefore targeting surveillance efforts at WNV-positive dead birds locations may aid in the reduction of WNV activity as well as help to drive additional control efforts. The dead bird program plays a significant role in the early indication of WNV activity within the District borders, but future work is needed to determine the dynamics between WNV-positive dead bird detections and how best to utilize the data operationally.

Methods

Dead bird collection

The California Dead Bird Surveillance Program was established in 2001 and expanded its reporting abilities in 2002 (McCaughey et al., 2003). In 2002, the California Department of Public Health (CDPH) established a public reporting system that included both a toll-free hotline and website for online reporting of dead birds. CDPH functioned as a call center to receive dead bird reports and dispatch information to the local Districts for the pickup and the processing of dead birds. This program is heavily utilized by SYMVCD and since the establishment of the program the District has conducted multiple public outreach campaigns to build awareness.

Dead bird sampling

American crows (*Corvus brachyrhynchos*) were sampled using the oral swab collecting method. This method is reliable for crows due to the high levels of virus they shed in their saliva but is unreliable on non-crow species (Padgett et al., 2006). For fresh or relatively fresh carcasses, a swab (Puritan, Guilford, ME; item #25-3000-H E30) is inserted into the buccal cavity and rotated back and forth several times to collect a robust sample. The swab tip then is placed into a tube of viral transport media (VTM) which contains Dulbecco's Modified Eagle Medium, fetal bovine serum to protect the virus and gentamicin, penicillin and amphotericin to inhibit the growth of bacteria and fungi.

Brain aspirates were collected from all other bird species. This method requires the carcass to be relatively fresh and intact as any intrusion of the cranium will allow air flow and impede suction of tissue into the syringe. A 16- or 18-gauge needle is placed on a 3ml syringe for tissue collection. To collect brain tissue, the needle punctures through the side of the skull and into the brain tissue. Once the syringe is inside the brain cavity, a side-to-side movement of the syringe can improve the amount of sample collected. Brain tissue samples were preserved in MagMAX lysis buffer (Life Technologies, Foster City, CA).

Maggots were collected from all species of birds when present. Any instar could be collected, but the 2nd instar was the easiest to collect and homogenize. The larger older instar maggots have a thicker integument making homogenization more difficult. Maggots with a visible gut load (a red spot in the maggot body) were preferred. In total 5-6 maggots were collected and preserved in MagMAX lysis buffer. To prevent any contamination between the sampling, single-use forceps were used for maggot collection.

Occasionally, dead birds were brought into the lablratory that were completely desiccated. To sample these birds, a wet swab approach was used. The swab handle was used to puncture the dry orbital cavity and create a route of entry into the cranium. The swab applicator tip was then dipped into MagMAX lysis buffer and inserted through the puncture and into the cranium through the orbital cavity. The inside of the cranium was swabbed with a rotating motion to maximize sample collection. In some cases, birds were brought into the laboratory missing their heads. In this situation, the carcass body cavity was swabbed, targeting the kidney area when possible. The sample applicator tip was preserved in MagMAX lysis buffer.

Sample processing and testing

The extraction process began by homogenizing all the maggot and brain aspirate samples. Two (5 mm) glass ball bearings were added to each sample, followed by homogenization for 3 minutes at 1060 cycles/minute in a Spex Sample Prep 8000D (Spex Sample Prep, Metuchen, NJ). The samples collected on swab applicator tips did not require homogenization, but each was vortexed prior to processing. All the dead bird samples were extracted using the 5x Viral kit (Life Technologies, Foster City, CA) and the MagMax 96-Express magnetic particle processor (Life Technologies, Life Technologies, Foster City, CA). The extracted RNA samples were analyzed by qRT-PCR with the QuantStudio 5 Real-time PCR system, using (TaqMan™ Fast Virus 1-Step Master Mix for qPCR) using previously published primers (Brault et al., 2015) to detect the presence of WNV, Saint Louis encephalitis virus, and western equine encephalitis virus. RT-PCR standards and blank controls were included for quality control.

Results and Discussion

Dead bird species trends over a 13-year period

The dead bird collection and testing data were analyzed over a 13-year period to identify the most common WNV-positive species within the District borders. Over the 13-year period, there were a total of 25 species of birds collected and tested. The seven most common species were American crow, California scrub-jay (*Aphelocoma californica*), yellow-billed magpie (*Corvus nutalli*), house finch (*Haemorhous mexicanus*), American robin (*Turdus migratorius*), northern mockingbird (*Mimus polyglottos*), and house sparrow (*Passer domesticus*). The percent positive varied by species as follows: American crow 1529 positive/2299 tested (66.5%), California scrub-jay 1020+/1932 (52.8%), yellow-billed magpie 333+/529 (64.2%), house finch 182+/500 (36.4%), American robin 104+/394 (26.3%), northern mockingbird 98+/393 (30.3%), and house sparrow 51+/304 (16.7%). Our District serves both Sacramento and Yolo Counties, and although the counties are remarkably similar, the human population density in Sacramento County was 1600 people per square mile whereas the Yolo County population density was 210 people per square mile. The total number of dead birds collected from each county reflected these human population density differences. Nearly 80% of all dead birds collected came from Sacramento County, reflecting the increased contact between dead birds and people that could report them.

Response to a WNV-positive dead bird

In 2023, a total of 44.1% of all dead birds collected were WNV-positive. The high number and the wide distribution of WNV-positive dead birds (Figure 1) provided an opportunity to refine the field response plan to WNV-positive dead birds. The mission of our District is to protect the public health from mosquito-borne viruses and the actions taken to accomplish this are a multi-tiered approach. The presence of a WNV-positive dead bird indicates WNV activity and triggers a two-part surveillance response plan including adult mosquito surveillance and larval mosquito control.

Adult mosquito traps are set in 3-4 locations within a 1.0-mile radius of the WNV-positive dead bird collection location. Sites are trapped with a single carbon dioxide baited encephalitis virus trap (EVS) and a single gravid trap (GT). This additional trapping assesses the abundance and WNV infection rate of local mosquito populations to decide what control interventions would be appropriate.

In addition to mosquito trapping, known aquatic mosquito habitats within a pre-determined radius, determined by the species of the dead birds, were inspected for mosquitoes. Three different radii were used based on the home ranges of the dead birds and the potential distance a WNV-infected bird would travel. For example, the house finch was assigned 0.25-mile radius of inspection and the American crow was assigned a 1.0-mile inspection radius, reflective of their larger flight range. Within each dead bird radius, control technicians inspected all potential mosquito sources and conducted larval mosquito control as necessary.

Conclusion

The dead bird surveillance program is an integral part of SYMVCD WNV surveillance program, because it is known that the WNV-positive dead birds are reliable indicators of WNV activity. Currently, WNV-positive dead birds trigger two specific actions. The first action was response trapping to assess vector abundance and WNV infection rates and the second is larval source surveillance and larvicide application as necessary to reduce mosquito populations.

Future work is needed to understand how WNV-positive dead birds and vector abundance and more specifically virus infection rates are related. This analysis will inform SYMVCD intervention efforts to best interrupt transmission cycles. Additionally, it is also necessary to understand the seasonal utility of the dead bird program. For instance, in the late spring/early summer a WNV-positive dead bird may be the first indication of virus activity in the area, but later in the season often dead birds and mosquito pools concurrently detect viral activity. Given, levels of routine and response trapping conducted by SYMVCD understanding the proportion of mosquito control efforts that are attributable

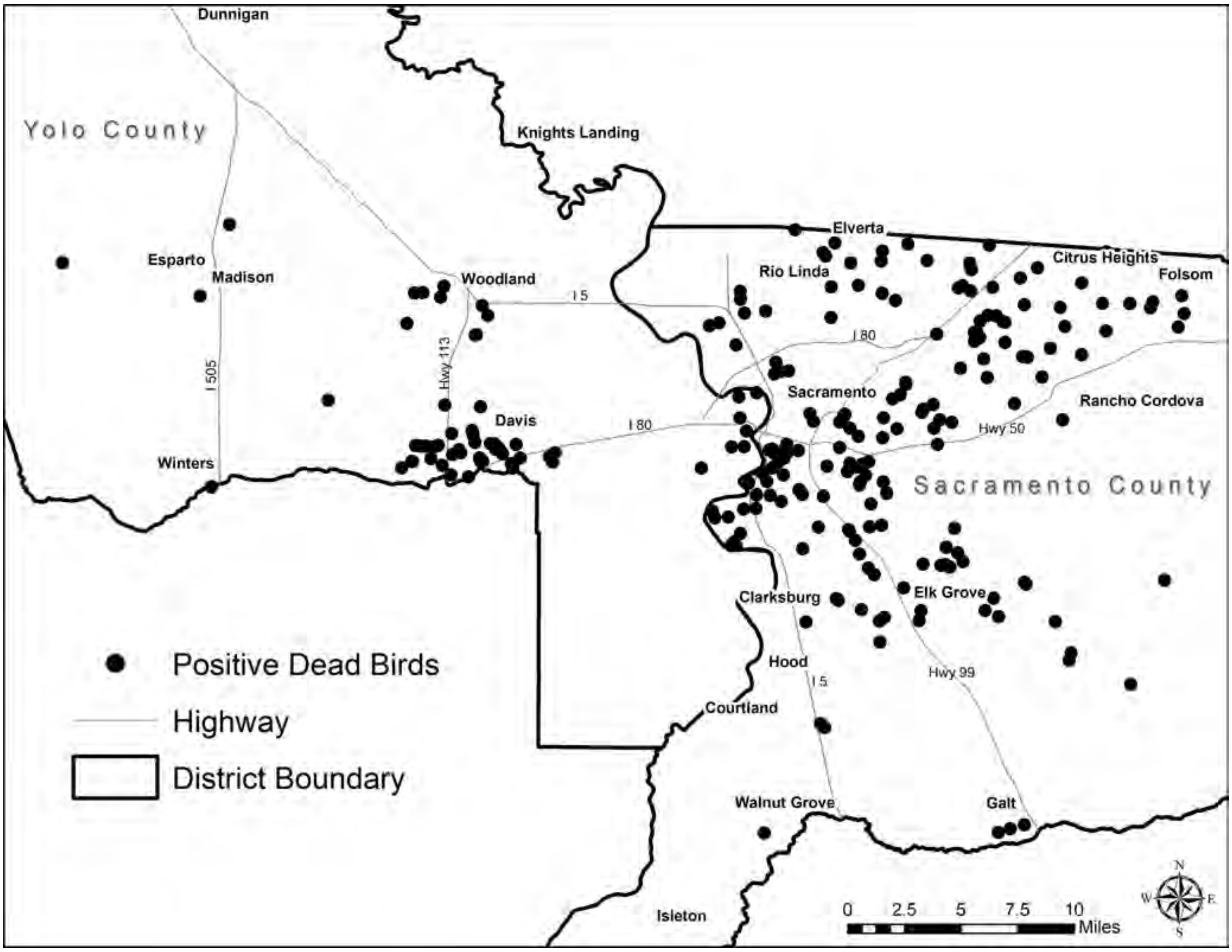

Figure 1.—Map showing the distribution of West Nile virus positive dead birds collected in 2023 from Sacramento and Yolo Counties.

to the dead bird surveillance program is important for deciding how best to allocate District resources.

Acknowledgements

We want to thank the Sac-Yolo MVCD Technicians for all their hard work collecting and organizing the dead birds brought into the District for testing.

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Enriching practical learning resources for public health entomology training

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Abstract

Training is a core pillar of the Pacific Southwest Center of Excellence in Vector-Borne Diseases' approach to enhance the capacity of the Southwestern United States to respond to threats from vector-borne pathogens that affect human health. We have made progress on four main projects focused on enriching practical learning resources for public health entomology: (1) Development of videos on trapping methods in collaboration with the MVCAC Laboratory Technologies Committee, (2) Development of a Vector-borne Diseases for Physicians module to be included in the Global Health Certificate through the UC Davis Medical Center, (3) Development of a hands-on and applied undergraduate medical entomology laboratory course to be taught at UC Davis for the first time in Fall 2024, and (4) Participation in a newly established Cross-Center Working Group on Innovative Approaches to Public Health Entomology Training alongside the CDC Centers of Excellence and Teaching, and Evaluation Centers to standardize curriculum and share resources. These four projects are expected to be completed by the end of 2024. The Pacific Southwest Center of Excellence in Vector-Borne Diseases continues to enrich practical learning resources for public health entomology training at multiple academic levels as well as for public health and vector control professionals.

The pivotal power of prudence when managing Mosquito and Vector Control money

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Abstract

Mosquito and vector control districts face multifaceted challenges, including the emergence of new diseases, invasive vector species, evolving insecticide resistance, and increasing public expectations. Efficient financial management is vital to address these challenges effectively. Adequate funding coupled with knowledge in financial management ensures that essential tasks such as control, surveillance, research, and outreach can be carried out consistently. Financial management encompasses budgeting, resource allocation, and strategic planning. A well-structured budget allows mosquito and vector control districts to allocate resources judiciously, prioritize activities, and respond promptly to disease outbreaks or environmental changes. Strategic financial planning ensures long-term sustainability, enabling districts to invest in research and technology to stay ahead of emerging threats. Adequate funding enables districts to hire and retain skilled personnel, acquire state-of-the-art equipment, and implement innovative control measures. It also supports community engagement and education initiatives, promoting public awareness and participation in vector control efforts. Financial management also plays a critical role in ensuring compliance with regulatory requirements and transparency in operations. Proper record-keeping and reporting are essential for accountability and stakeholder trust, fostering cooperation among local governments, public health agencies, and the communities served. Effective financial management is the linchpin of successful mosquito and vector control districts. It enables districts to respond to dynamic challenges, deliver high-quality services, and maintain the public's trust. As the landscape of vector-borne diseases continues to evolve, robust, thoughtful and prudent financial management remains imperative for safeguarding public health and creating resilient mosquito and vector control systems.

Comparison of RT-PCR results for mosquito testing at various simulated shipping temperatures

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Abstract

Over 30 California mosquito control agencies routinely ship mosquito pools to the UC Davis Arbovirus Research and Training (DART) laboratory for arboviral testing by RT-PCR. Shipping requires packing the mosquito pools inside an insulated box packed with dry ice to keep the mosquitoes at ultra-low temperatures throughout shipment. The need for dry ice in shipments results in additional costs and effort required for its procurement, as well as additional requirements for labeling and handling. In this study, we compared RT-PCR test results for West Nile virus (WNV)-positive mosquito homogenates under a range of simulated shipping conditions.

Homogenates from previous mosquito pools that were WNV-positive by RT-PCR were thawed, combined and vortexed to create a set of 20 mosquito homogenates of known initial Ct value. Each of the homogenates where then divided into a series of tubes, resulting in equivalent sets of 20 mosquito homogenates corresponding to each experimental condition and simulated shipping time (0, 24, 48, and 72 hours). Four experimental conditions were considered: −80C freezer (positive control), insulated shipping box with dry ice, insulated shipping box with blue ice gel packs, or insulated shipping box without a cooling agent. After packing, insulated shipping boxes were sealed to simulate shipping conditions, then held at ambient laboratory temperature for up to 72 hours. At the pre-determined times of 0, 24, 48, and 72 hours, samples were retrieved and tested from each experimental condition, and Ct values were compared across experimental conditions and time points.

As expected, simulated shipments with dry ice resulted in Ct values that approximated those of the −80C freezer. Simulated shipments on blue ice gel packs achieved Ct values that were only slightly higher (< 1-2 Ct units) than those of dry ice,. The highest Ct values were associated with room-temperature shipments without any added cooling agent (up to 4.8 Ct units higher than dry ice after 72 hours). This study's findings will be used in relation to recommendations for shipping mosquito pools to DART in the future.

Cybersecurity Risk Management

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Abstract

This presentation explored cybersecurity risk management in today's technology landscape, emphasizing its importance and the benefits of implementation. It outlined the cybersecurity risk management lifecycle, focusing on identifying, assessing, remediating and reviewing risks. The advantages of such programs, including enhanced security, better ROI on IT spending, regulatory compliance, and cybersecurity insurance requirements were discussed. Also discussed were risk assessment through identifying threats, vulnerabilities, and impacts, while promoting collaboration and support across agencies in cybersecurity efforts. It closed with a call to action to tackle the initial steps to implement a risk management plan, and attendees to free resources available from the MVCAC Information Technology Committee, the Municipal Information Systems Association of California, the national Cybersecurity and Infrastructure Security Agency and the National Institute of Standards and Technology Computer Security Resource Center.

VectorSurv Cybersecurity Update

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Introduction

More than 60 mosquito and vector control agencies across California use VectorSurv to manage surveillance data, generate reports, and make data-based decisions (VectorSurv Development Team 2024). VectorSurv users must have reliable access to their data and the ability to control who has access to it. This means ensuring that data are available when needed, and that data are protected from malicious 'actors' or other cybersecurity threats. In today's evolving cybersecurity threat landscape, it is necessary to have a process for identifying and responding to information security risks. VectorSurv is adopting the processes and guidelines defined in NIST Special Publication 800-53, Security and Privacy Controls for Information Systems and Organizations (National Institute of Standards and Technology, 2020). The process we are using to implement the NIST 800-53 standard is described below.

Methods

We began by engaging the UC Davis Information Security Office to assist in performing a security assessment. First, we analyzed the VectorSurv system, use cases, and usage to determine an appropriate baseline set of controls from the NIST framework. We defined VectorSurv as two separate systems for this project: (1) the VectorSurv web application and (2) the VectorSurv hardware environment. The different profiles of these two systems meant they each had a unique control baseline.

After identifying the appropriate controls, we assessed our current security activities through a series of interviews with the information security office, developers, and administrators. Through this assessment we were able to align our information security activities with the controls defined in NIST 800-53 to identify strengths and weakness in our security posture. Finally, we created the initial documentation and began creating a plan for implementing additional security controls. This documentation will serve as a foundation for adapting and improving VectorSurv's security into the future.

Results and Discussion

After completing our initial information security assessment, we generated an inventory of controls to protect VectorSurv data, and a plan for adopting additional controls. We are in the process of creating security policies to define and communicate the controls in place at any time. These policies indicate activities performed and how they relate to the controls recommended by the NIST 800-53 framework. Examples of activities include providing recurring developer training, performing code reviews, verifying the security of tools, keeping tools updated, securing computing hardware, maintaining firewalls to monitor and control network traffic, and following best practices for backing up data.

Another important element of the NIST framework is the risk assessment process. Our risk assessment process involves assessing threats to VectorSurv periodically to assess how our controls and activities address current information security needs. These security policies, activities, and controls will be reviewed when necessary and updated annually to ensure that our security activities adapt to the evolving security landscape.

In addition to system-wide security policies and procedures, it is imperative that all VectorSurv users develop and practice good information security habits. Many cybersecurity attacks, such as phishing or other social engineering attacks, begin by obtaining a user's authentication credentials, through deceit or more sophisticated methods, so that they can access data without arousing suspicion or needing to compromise the security of a system directly. VectorSurv users can help to protect their data by ensuring that usernames and passwords are unique to a single person, keeping passwords secret, and using passwords that are unique for every system they use. It is also important for agency managers in VectorSurv to regularly review all user accounts who have access to their agency's data and disable any accounts that no longer need access.

Conclusions

By adopting and implementing the Security and Privacy Controls for Information Systems and Organizations defined in NIST Special Publication 800-53 as our guideline for information security, the VectorSurv team is working to reduce the risk and impact of information security threats. Our commitment to the NIST 800-53 framework includes ongoing review and improvement, ensuring that we will continue to advance and update our security posture to address emerging threats.

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 CalSurv's mission to strengthen the evidence basis for vector-borne disease control. CalSurv is made possible by funding support from the State of California and Epidemiology and Laboratory Capacity for Infectious Diseases Cooperative Agreement number NU50CK000539 from the US Centers for Disease Control and Prevention.

We also thank Julio Cardenas and Cheryl Washington at the UC Davis Information Security Office for their help and guidance with assessing our security needs, implementing the NIST 800-53 controls, and their continued assistance in strengthening our information security.

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Multiple insecticide resistance in *Culex tarsalis* populations from Coastal, Northern, and Central California

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Abstract

In California, *Culex tarsalis* are the predominant mosquito vector for West Nile virus in rural and agricultural areas. Resistance to permethrin, deltamethrin and malathion was assessed in *Cx. tarsalis* populations across three broad regions of agricultural and urban importance in California: San Francisco Bay Area, Sacramento Valley, and San Joaquin Valley. Bottle bioassays using diagnostic times and diagnostic doses provided by the CDC Bottle Bioassay Kit were conducted by Vector-Borne Disease Section, California Department of Public Health, staff in cooperation with local vector control agencies. This presentation summarized the results of the regional resistance survey for *Culex tarsalis*.

Pyrethrin insecticide resistance expressed in laboratory and field settings with *Culex tarsalis* populations from Sacramento and Yolo counties

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Abstract

The overarching goal of our project is to bridge laboratory resistance bioassays to field trials with *Culex tarsalis* with known resistance to pyrethrins. By assessing the relationship between laboratory bioassay results, survivorship within caged field trials, and percent reduction obtained from concurrent monitoring of field population abundance, a better gauge of control efficacy against resistant populations can be obtained.

In the laboratory setting, female *Cx. tarsalis* mosquitoes undergo resistance testing using three bioassays with pyrethrin: the standard CDC bottle bioassay (LT50), a topical assay (LD50), and a novel individual mosquito bottle bioassay (an individual time to knockdown). Additionally, populations were characterized for kdr mutations and detoxifying enzyme levels to provide insight into the mechanisms behind their resistance.

The field component of the project focused on semi-field trials using sentinel cages, monitoring the abundance of field populations to estimate percent reduction, and analysis of wild mosquito population age stcrtucture to assess the overall control efficacy of Pyronyl 525 Oil Concentrate (active ingredients: 5% pyrethrins, 25% piperonyl butoxide). Wild, female CO2 trapped *Cx. tarsalis* were transferred to sentinel cages and exposed to a pyrethrin insecticide treatment via aerial treatment in the field. Time to knockdown was observed over time, with little difference observed between known susceptible and wild populations. Concurrently, abu+ndance before and after insecticide application was used to monitor percent reduction in population size. Pre- and post-spray samples of females were collected from the CO_2 traps and aged via NIRS (near-infrared spectroscopy) using newly developed aging models. By determining the population age structure, and comparing pre- and post-spray in abundance, a better understanding of the effects of insecticide treatment on resistant *Cx. tarsalis* populations may be achieved.

This project provided background for future research into control efficacy and making laboratory tests more useful in guiding vector control districts through treatment implementation with resistant populations. Rather than simply knowing that a population has a percentage with resistance to pyrethrins, the impact of that resistance on population response to treatment would be critical information.

Testing the novel adulticide ReMoa Tri against rice field mosquitoes in northern California

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Introduction

Northern California's rice fields provide extensive larval habitat for *Culex tarsalis*, a primary vector of West Nile virus, and *Anopheles freeborni*, a significant pest of humans. The combination of large rice acreages and small to medium-sized human population centers creates a situation where limited funding prevents complete control of mosquitoes in the larval stage. The resulting reliance on mosquito adulticides to protect areas of human habitation leads to the potential development of adulticide resistance in mosquito populations.

Rotation of insecticide active ingredients and modes of action is often suggested as a method for combating resistance development (Hemingway and Ranson 2000). However, the effectiveness of mosquito adulticide rotation is hampered by a dearth of available modes of action with currently only pyrethroids and organophosphates registered in California for adult mosquito control. An upcoming alternative, ReMoa Tri (Valent BioSciences LLC), is an adulticide with three active ingredients including a novel mode of action for mosquito control (Lucas et al. 2024, Unlu et al. 2024). A bacterially produced ingredient, abamectin, functions by modulating glutamate-gated chloride channels leading to insect paralysis. This is combined with fenpropathrin (a Type II pyrethroid) and C8910 fatty acid. The mode of action of C8910 is undetermined though there is some evidence that it may affect the insect respiratory system (Samuel et al. 2015) and may synergize pyrethroids (Ramadan et al. 2022).

Personnel from Sutter-Yuba MVCD in collaboration with representatives from Valent BioSciences performed a field trial of ReMoa Tri (https://www.valentbiosciences.com/publichealth/products/remoa-tri-faqs/) under an experimental use permit using local mosquitoes. Collections of *Cx. tarsalis* came from Pierce Road near Yuba City where populations have been monitored regularly for resistance for many years. Pierce Road *Cx. tarsalis* populations regularly show some resistance to organophosphates and stronger resistance to pyrethroids in bottle bioassays performed by the district and by other researchers (Hughes 2017) while field cage tests give mixed results with evidence for pyrethroid resistance in some trials. Collections of *An. freeborni* came from Robbins, CA where adulticide application is frequent but less data on resistance is available.

Methods

A field cage trial was performed on August 1, 2023 in a 'bare ground' area near rice fields north of Robbins, CA. Two replicate applications were performed consecutively at dusk with a truck-mounted ULV fogger (Clark Promist Dura, < https://www.clarke.com/mosquito-equipment/application-equipment/promist-dura-ulv-sprayer/ >) passing at 10 mph upwind of a line of cage stations. The fogger flow rate was calibrated to 4.5 oz/minute resulting in an application rate of 0.75 oz/acre of undiluted ReMoa Tri. The sprayer was turned on a quarter mile before passing the line of field cages and turned off a quarter mile after passing. Field cages were collected 20 minutes after application and stored in a cooler with access to cotton soaked with 10% sugar water.

Field stations were placed in a single line with a station located at 100, 200, 300, 500 and 750 feet from the path of the truck sprayer. Each station consisted of three mosquito field cages on a tripod with a wind vane and a separate tripod supported a Leading Edge (leateam.com) spinner with two Teflon-coated 3 mm glass rods. Tinopal UV dye was added to the ReMoa Tri to allow droplet analysis with ADrop (https://www.valentbiosciences.com/). Mosquito field cages contained 15 field *An. freeborni* captured in Robbins, CA, 20–25 field *Cx. tarsalis* captured from Pierce Road (southwest of Yuba City, CA), or 20–25 laboratory *Cx. tarsalis* (3–7 days old) from the susceptible KNWR colony maintained at Sutter-Yuba MVCD. Pierce Road was chosen as a source of resistant *Cx tarsalis* because previous bottle-bioassay data has shown pyrethroid resistance at this location. A single control station with all three cages was placed upwind from each application.

Following completion of the first application all cages and rods were replaced with fresh material. After both applications were complete, cages were transported back to the district and placed on a table to count the number of mosquitoes knocked down. Mosquitoes were considered knocked down if they were unable to stand (but may still move legs or wings). Counts were taken about 1, 16, 32, 40 and 56 hours after the field trial covering a timeframe comparable with other tests of ReMoa Tri (Lucas et al. 2024, Unlu et al. 2024). Sugar water was added to cotton balls twice a day.

Table 1.—Droplet analysis using ADrop of Teflon-coated 3 mm rods placed on spinners at each field station during two consecutive applications. Volume median density (VMD) and number median diameter (NMD) are measured in microns (μ).

Station Distance	Total Droplets	VMD	NMD	Density (mm^2)
Run 1				
100 ft	268	20	9	37
200 ft	160	17	7	22
300 ft	106	21	8	17
500 ft	75	17	8	14
750 ft	56	21	6	10
Run 2				
100 ft	108	18	9	12
200 ft	180	19	8	28
300 ft	109	19	8	16
500 ft	99	20	7	14
750 ft	73	16	9	9

Results and Discussion

During the first application the wind was 3.4–6.4 mph from the south and temperature was 69 F while wind during the second application (approximately 45 minutes later) was 3.6-5.0 mph with a temperature of 66 F. Droplet analysis of the Teflon-coated rods (Table 1) resulted in a droplet density ranging from 9 to 37 drops per mm^2 and a volume median diameter (VMD) between 16 and 21 μ. In general droplet density tended to decrease with distance, with the notable exception of the 100 foot station in application 2 which was unexpectedly low (Table 1). In general spray droplet numbers were comparable between applications.

In the field cages, upwind controls had low mortality with three colony mosquitoes knocked down in each of first and second application controls by the final 56 hour time point. None of the wild-caught control mosquitoes were knocked down (Table 2). In contrast 100% of colony *Cx tarsalis* mosquitoes were knocked down at the 1 hour after treatment (HAT) time point in both applications.

Overall, high mortality was seen in treatment cages containing wild-caught mosquitoes, particularly by the final time point. In the first application, for instance, all cages out to the 750 foot station reached 89-100% KD by 56 HAT. Knockdown percentage was higher in the first application than in the second in both wild-caught species, though the cause is unknown. Excluding the anomalous 100 foot station reading in the second application, droplet densities and VMDs were similar between applications while environmental measurements recorded similar wind speeds and a three degree lower temperature during the second application. Possibly an unmeasured environmental factor or change in wind at the moment of application contributed to the differences in outcomes between applications. When the two applications were averaged together, high mortality (85–100%) was seen across species and station distances at the 56 HAT time point (Fig. 1).

Table 2.—Percentage knockdown of mosquitoes in field cages placed at various distances (in feet) from the ReMoa Tri application truck which passed in two consecutive applications. Knockdown percentages are given for 16, 32 and 56 hours after treatment (HAT).

	Run 1			Run 2		
Cage distance	16 HAT	32 HAT	56 HAT	16 HAT	32 HAT	56 HAT
Susceptible Colony *Culex tarsalis*						
100	100	100	100	100	100	100
200	100	100	100	100	100	100
300	100	100	100	100	100	100
500	100	100	100	100	100	100
750	100	100	100	100	100	100
Control	10	10	15	0	4	12
Wild-caught *Culex tarsalis*						
100	100	100	100	54	54	77
200	88	88	100	92	88	96
300	78	81	89	52	67	81
500	90	90	100	52	74	87
750	83	83	96	65	65	85
Control	0	0	0	0	0	0
Wild-caught *Anopheles freeborni*						
100	100	100	100	33	40	87
200	100	100	100	80	87	100
300	93	100	100	47	47	87
500	73	87	93	67	73	87
750	87	93	100	57	64	93
Control	0	0	0	0	0	0

Among the various environments in which Sutter-Yuba MVCD performs cage trials, truck-based spray over the agricultural fields tends to yield the highest mortality and lowest resistance detection in field cages. The addition of environmental complexity (trees, houses, etc.) or distance to the spray source (airplane, for example) can increase the differences seen between mortality of wild-caught and colony mosquitoes. In the present study, although mortality was high across sampling stations, many stations had some surviving wild-caught mosquitoes, while all colony

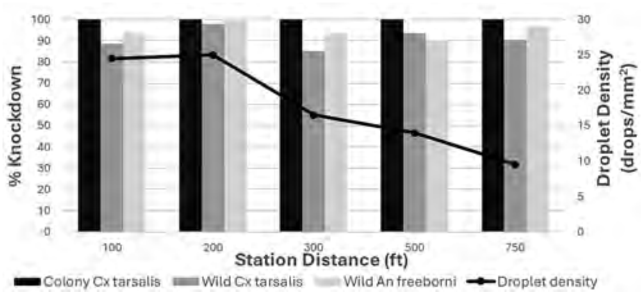

Figure 1.—Percentage knockdown after 56 hours of mosquitoes in cages placed at various distances from the Remoa Tri spray source compared with the droplet density measured on Teflon-coated 3 mm rods placed on spinners. Both knockdown percentage and droplet density are averaged across two replicate spray applications.

mosquitoes were quickly knocked down. This indicates that some resistance to ReMoa Tri is currently present in wild mosquito populations, possibly due to the previously documented pyrethroid resistance and/or cross-resistance with the new mode of action. Given this low-level resistance, ReMoa Tri may be well suited for use as one of several formulations which may be frequently rotated to enhance control. Additional field trials directly comparing ReMoa Tri with better studied formulations of pyrethroids may elucidate the situations and locations where ReMoa Tri will be most effective at the Sutter-Yuba MVCD.

Conclusions

A field trial of the novel adulticide ReMoa Tri under favorable field conditions resulted in high mosquito mortality up to 750 feet from the spray source. This trial showed that the new formulation was potentially capable of reducing numbers of rice field mosquitoes but also demonstrated that low-level resistance may already exist. A major barrier to combating adulticide resistance is the low number of registered modes of action available and the addition of another tool would strengthen insecticide rotation strategies.

Acknowledgements

John Holick and Haley Johnson of Valent BioSciences were instrumental in designing and performing the field trial and Dennis Candito of Azelis Agricultural and Environmental Solutions aided in field trial execution.

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Beyond Chemical Standards: can better agreement between the bottle bioassay and operational field efficacy be achieved?

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Introduction

Currently, chemical intervention is a key strategy used to control insect populations, mitigate negative effects on agriculture, and protect the health and quality of human and animal life. Over time, changes in environmental regulations and the development of insecticide resistance have restricted the availability of chemical tools and threatened the efficacy of remaining products. Mosquito and vector control agencies have used sentinel field cages to verify the effectiveness of adult mosquito control applications for decades (Rogers et al., 1957). More recently, the CDC Bottle Bioassay (BBA) has been added as a method to monitor the presence of chemical resistance in adult mosquito populations that impact adulticide efficacy (Brogdon and McAllister, 1998). Unfortunately, the data obtained from the two methods are not always in concordance. Previous work done at the Sacramento -Yolo Mosquito and Vector Control District using colonized susceptible and colonized resistant adult mosquitoes suggested that there was better correlation between field and BBA results when the formulated product was used in the bottle (Dritz et al., 2020). The objective of the current study was to further explore our previous results by performing a matched BBA and field cage trial that included both field-collected and colony reared susceptible mosquitoes that were exposed to two different classes of chemical standard and formulated product.

Materials and Methods

Mosquitoes

Culex pipiens were collected from Fogg Road in Sacramento County, California using both CO_2-baited and gravid traps. Gravid females were allowed to oviposit, and a population of Fogg Rd *Culex pipiens* was established for this study. *Culex tarsalis* were collected from Vic Fazio Wildlife Refuge, Yolo County, CA using CO_2-baited traps as needed and were not maintained in colony. The two susceptible colonies used were CQ1 (McAbee et al., 2004) *Culex pipiens* complex (hereafter, *Cx. pipiens*) and KNWR *Culex tarsalis* (Reisen et al., 2005).

Semi-field trial

An open field was used to conduct the field application. A transect grid was established so that application was evaluated in triplicate at 100, 200, and 300 ft from the spray path. Fyfanon EW (FMC, Philadelphia, PA) and Deltagard (Bayer, Pittsburg, PA) were applied at 1.5 fl oz/acre and 0.67 fl oz/acre, respectively. The applications were made on the same night using two different trucks equipped with identical ULV Foggers (London Fogger, Minneapolis, MN; model # XKE), driving at 10 mph. At each evaluation point four sentinel cages (Townzen and Natvig, 1973) each containing 20 female mosquitoes from CQ1, Fogg Rd *Cx. pipiens*, KNWR, and Vic Fazio *Cx. tarsalis* populations were affixed to a cage vane that self-oriented into the wind (Vessey et al., 2007). Control cages (three for each population) were set 45 minutes prior to applications to allow exposure to field conditions, then collected prior to spray trials. For each application a new set of cages was deployed, the application was made, then the cages were left undisturbed in the field for 30 min prior to collection. Cages were checked for mortality at set up (0 hour), pick up (0.5 hour) and 12 hours post-spray.

Bottle bioassays

The CDC BBAs (Brogdon and Chan, 2012) were performed using both formulated products (Fyfanon EW and Deltagard) and technical grade chemical standards (malathion and deltamethrin) on matched susceptible and field populations. Fogg Rd *Cx. pipiens* were compared with CQ1 and Vic Fazio *Cx. tarsalis* with KNWR, the same populations used in the semi-field trials. Twenty-five females were placed in each bottle and four replicates of each chemical plus four acetone only control bottles were used per mosquito population. The deltamethrin chemical standard (N-11579-250mg Chem Service Inc., West Chester, PA) and Deltagard were used at the dose of 22ug/bottle. The malathion chemical standard (N-12346-100mg Chem Service Inc., West Chester, PA) and Fyfanon were used at 400 mg/bottle dose. Counts were performed every 5 minutes for the first 15 minutes and every 15 minutes thereafter for a total of 180 minutes. If mortality in the acetone control bottles was greater than 3%, Abbott's formula was used to correct the findings (McAllister and Scott, 2019).

Results and Discussion

Post-spray counts (12 h) of sentinel cages exposed to Deltagard (Figure 1A) showed an overall mean mortality of

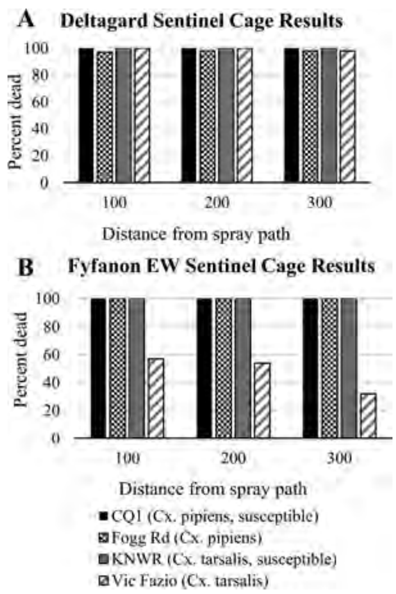

Figure 1.—Field sentinel cage mean mortality by distance in feet from spray path collected 12 h post-application for both Deltagard (A) and Fyfanon (B).

100% in both susceptible populations [CQ1 (9 sentinel cages, n=164), KNWR (9 sentinel cages, n=181)], indicating that the spray cloud moved across all sentinel cage locations during this application. The overall mean mortality of the Fogg Rd *Cx. pipens* and Vic Fazio *Cx. tarsalis* was 97.7% (9 sentinel cages, n=176) and 99.4% (9 sentinel cages, n=178), respectively. These results indicated that Fogg Rd. *Cx. pipiens* and Vic Fazio *Cx. tarsalis* were susceptible to Deltagard.

Post-spray counts (12 h) of the sentinel cages exposed to Fyfanon EW (Figure 1B) showed overall mean mortality of 100% in CQ1 (9 sentinel cages, n=178), KNWR (9 sentinel cages, n=180), and Fogg Rd *Cx. pipiens* (9 sentinel cages, n=187). These results indicated that Fyfanon EW also moved across all sentinel cage locations and that the Fogg Rd *Cx. pipiens* were susceptible to this product. In contrast, the Vic Fazio *Cx. tarsalis* showed an overall mean mortality of 47.4% (9 sentinel cages, n=173), indicating that Fyfanon EW had reduced effectiveness for this population.

The BBA results for Deltagard and deltamethrin appear in Figure 2 A-B. Mortality in the acetone control bottles was less than 3% for all assays and Abbott's formula was not required. For *Cx. pipiens* the diagnostic time (time at which all susceptible mosquitoes were dead or knocked down and unable to stand) was 30 min for deltamethrin (4 bottles, n=99) and 15 min for Deltagard (4 bottles, n=99). At the diagnostic time the percent mortality in the Fogg Rd *Cx. pipiens* was 17.3% (4 bottles, n=98) for deltamethrin and 52.5% (4 bottles, n=99) for Deltagard. Per the CDC BBA guidelines (McAllister and Scott, 2019) mortality $<90\%$ at the diagnostic timed implies resistance. However, all the mosquitoes in the Deltagard treated bottles were dead by 120 min, whereas only 85.7% of the mosquitoes were dead in the deltamethrin bottle at the end of the 180 min assay.

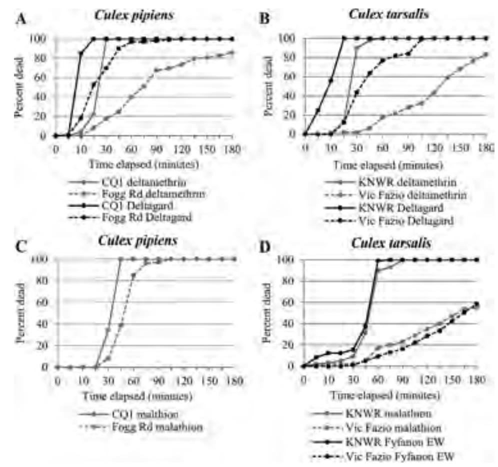

Figure 2.—Bottle bioassay overall mean mortality by exposure time: CQ1 and Fogg Rd *Culex pipiens* were tested against deltamethrin and Deltagard (A), and malathion (C); KNWR and Vic Fazio *Culex tarsalis* were tested against deltamethrin and Deltagard (B), and malathion and Fyfanon EW (D).

This indicates that Deltagard was more effective than deltamethrin at overcoming resistance factors. The pattern in *Cx. tarsalis* was similar. The diagnostic time for deltamethrin (4 bottles, n=99) was 60 min and 15 min for Deltagard (4 bottles, n=97). At the diagnostic time 17.5% of the Vic Fazio *Cx. tarsalis* population was dead in the deltamethrin bottles (4 bottles, n=97) and 12.1% in the Deltagard bottle (4 bottles, n=99), indicating the development of resistance to deltamethrin in these populations. However, similar to the results for Fogg Rd *Cx. pipiens,* all the mosquitoes in the Deltagard bottle were dead at 120 min, but at the end of 180 min, mortality in the deltamethrin bottles was at 83.5%.

The BBA results for malathion and Fyfanon EW appear in Figure 2 C-D. For *Cx. pipiens* the diagnostic time for malathion (4 bottles, n=99) was 45 min. While mixing the Fyfanon EW with acetone, a miscibility issue was detected. The Fyfanon EW label states that the product "may be used undiluted or diluted with water only." Because of this issue, the Fyfanon EW BBA was compromised, and results are not shown. Plans are underway to repeat this portion of the study utilizing methodology that was perfected and applied to our *Cx tarsalis* formulated product BBA.

Because Fyfanon EW is miscible in water, alternative substances that most closely mimic water were explored. The product went into solution using a 2 to 25ul solution of two hundred proof ethanol and Fyfanon EW. This was added to the appropriate amount of acetone to prepare a working solution which was used to coat bottles according to the CDC protocol. The *Cx. tarsalis* diagnostic time was 90 min for malathion (4 bottles, n=99) and 75 min for Fyfanon EW (4 bottles, n=98). At the diagnostic time the Vic Fazio *Cx. tarsalis* had 23.0% (4 bottles, n=100) mortality in the malathion bottles and 13.1% (4 bottles, n=99) mortality in the Fyfanon EW bottles. At the end of the assay time (180 min) there was 55.0% mortality in the malathion bottles and 58.6% mortality in the Fyfanon EW bottles indicating high levels of resistance to malathion in the Vic Fazio *Cx. tarsalis*. Because the malathion performance in the BBA (Figure 1D) was so similar to that of Fyfanon EW (when non-standard methodology was used to enhance miscibility of this product in acetone), there seems to be no advantage to use of formulated product in this case.

When the sentinel cage data was compared to the BBA data, some inconsistencies were observed. Bottle bioassays indicated that resistance was present to technical grade malathion and deltamethrin in both Fogg Rd *Cx. pipiens* and Vic Fazio *Cx. tarsalis*. However, not all BBA data aligned with the semi-field trial results.

Fogg Rd *Cx. pipiens* and Vic Fazio *Cx. tarsalis* mortality was nearly 100% in sentinel cages exposed to Deltagard (Figure 1A), indicating that these species were susceptible to the formulated product under application conditions. Bottle bioassays conducted with Deltagard showed delayed mortality curves for field populations compared to susceptible colonies, but the field populations attained 100% mortality before the end of the assay. This contrasted to bottles prepared with deltamethrin where field populations failed to attain 100% mortality. Bottles prepared with Deltagard were more indicative of sentinel cage outcomes than the bottles prepared with technical grade deltamethrin; however, the percent mortality at the diagnostic time would have underestimated the effectiveness of the product.

Comparisons of the sentinel cages exposed to Fyfanon EW (Figure 1B) and the BBA prepared with malathion and Fyfanon EW were impacted by the lack of miscibility of Fyfanon EW in acetone. However, since mortality curves between malathion and Fyfanon were so similar when miscibility of Fyfanon EW was enhanced, only the malathion BBA data was used for comparison to sentinel cage data. As with Deltagard, 100% mortality in BBA at 180 minutes was indicative of susceptibility in sentinel cage trials. The Fogg Rd *Cx. pipiens* had 100% mortality after 180 min in malathion bottles and 100% mortality in sentinel cages following exposure to Fyfanon EW in the field. In contrast, the Vic Fazio *Cx. tarsalis* had 55.0% mortality at 180 min in the BBA and a mean mortality in sentinel cages of 47.6%.

Overall, the percent morality of field populations at the diagnostic time was not a good indicator of pesticide performance in the field, whereas the percent mortality at the completion of the assay (180 min) more closely aligned with sentinel cage results. Not all protocols continue to observe mortality for 180 min and may conclude either at the diagnostic time or at 120 minutes. In this study BBA outcomes were similar at 120 and 180 min. Populations that had delayed mortality curves, but attained 100% mortality, did so by 120 min. However, the populations that did not have 100% mortality had greater mortality at 180 min than at 120 min. More study is needed to compare BBA outcomes to semi-field trials to determine whether this pattern holds true with all pesticides. However, we do know that mortality at diagnostic time is not a good indicator of how well a formulated product will perform in a semi-field trial.

Conclusion

Bottle bioassay results conducted with the deltamethrin chemical standard showed decreased mortality compared to BBAs performed with Deltagard formulated product and were not representative of semi-field trial outcomes. The Safety Data Sheet (SDS) for Deltagard indicates the composition is 98% undisclosed proprietary ingredients. In comparison, results from our *Cx. tarsalis* BBA comparing the Fyfanon EW formulated product with adjusted miscibility for acetone against the malathion chemical standard showed almost no difference in time mortality curves between formulated product and chemical standard. The Fyfanon EW SDS does not indicate the product contains undisclosed ingredients. Our study reinforces the possibility of misleading resistance profile data from the BBA if chemical standards alone are used to evaluate adult mosquito resistance to commercial products which contain undisclosed proprietary ingredients. Including formulated product in such cases provides superior data for program planning purposes. However, our data indicates that the performance of those commercial products which do not contain undisclosed ingredients may be accurately represented by use of a chemical standard in BBAs. Inclusion of both wild and susceptible mosquitoes provides better data resolution and where possible, field cage studies coupled with BBAs provide a more complete picture of adult mosquito resistance.

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Is malaria knocking on California's door?

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Abstract

Locally transmitted cases of malaria in Florida and Texas in summer 2023 made national headlines and prompted public health concerns. With a history of malaria outbreaks and the presence of competent mosquito vectors in California, it is possible for travel-associated cases to serve as sources of malarial parasites and initiate local transmission. In response to concerns about malaria transmission risk in California, we summarize demographic characteristics and travel history of cases of malaria reported in the state between 2013 and 2022. To assess risk of local transmission, we examined the degree of overlap between cases' county of residence, the geographic range of competent mosquito vectors, and the historical occurrence of local transmission. This evaluation informs key messages used to respond to inquiries about the possibility of local malaria transmission in California.

Climate change and mosquito control: The effect of atmospheric river events in California's Central Valley

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Abstract

Atmospheric rivers (ARs) are a type of storm that produce 50 percent of California's water supply and are responsible for 90 percent of the state's floods. As the name implies, they are like rivers in the sky. These elongated plumes of moisture carry saturated air from the tropics to higher latitudes and deliver large amounts of precipitation in the form of either rain or snow. In the winter of 2023, California experienced 12 of these extreme weather events which led to extensive flooding in areas that have become accustomed to severe drought. In March of 2023, both State and Federal Disaster Declarations were executed to assist communities impacted by flooding, snow, mudslides, avalanches, and debris flows that resulted from storms. Over roughly 3 weeks some parts of the state received 2-3 feet of rain and many communities struggled with flooding as water was diverted away from "towns" and into the once dry Tulare Lake basin which was previously recognized as the largest body of fresh water west of the Mississippi. The sudden reappearance of Tulare Lake, which was drained for farmland in the late 1800s, caused hundreds of millions of dollars in agricultural losses and the emergence of mosquitoes in unprecedented numbers. With assistance from the California Department of Public Health (CDPH), Vector Disease Control International (VDCI) was called in to assist vector control agencies in Tulare and Kings counties with aerial larval and adult mosquito control operations. This presentation covered the logistics involved with this emergency response, touched on climate change, and highlighted the importance of collaboration when dealing with unprecedented mosquito control issues.

Determining the identification and distribution of *Anopheles hermsi* and *Anopheles freeborni* using DNA barcoding techniques

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Introduction

Accurate identification of mosquito species is crucial for assessing disease risk and designing effective control strategies. However, cryptic species, which are closely related complexes indistinguishable by morphological traits but genetically distinct, can complicate species identification. This challenge is particularly significant among medically important arthropods such as anopheline mosquitoes, which are vectors of malaria parasites (Zhong et al., 2020). This study aimed both to evaluate genetic barcoding as a practical method to accurately identify two cryptic anopheline malaria vectors, *Anopheles freeborni* and *Anopheles hermsi*, and to elucidate the distribution of these two species in California.

Methods

Adult mosquito specimens were requested from vector control districts and other agencies with a history of *Anopheles freeborni* and *Anopheles hermsi* trap collections in VectorSurv. Samples were received from all regions of California and Salt Lake City, Utah. Mosquitoes were photographed, and DNA was extracted and analyzed using PCR amplification of the cytochrome oxidase1 (CO1) (Reeves et al. 2020) and internal transcribed spacer 2 (ITS2) (Collins et al. 1990) loci. Species identifications were confirmed with the use of NCBI's Basic Local Alignment Search tool (BLAST) and through phylogenetic analysis using a maximum likelihood approach.

Results and Discussion

Analyses of the ITS2 locus resulted in a clear delineation between the sister taxa, however, the CO1 locus failed to do so. This result is consistent with previous papers, showing the CO1 locus unable to separate recently diverged species (Carter 2019). These molecular results will assist in the clarification of the geographical distribution of both species in future studies. Here we note that despite previous research indicating *Anopheles freeborni* constricted to the Southern region of the state and *Anopheles hersmi* in the Northern (Baker and Kitzmiller 1963, Barr et al. 1988), an overlap of both species occurs in the San Francisco Bay Area (Fig. 1).

Conclusion

While CO1 is commonly used to differentiate many species (Hebert et al. 2003), the application of this locus for the delineation between the sister taxa, *Anopheles freeborni* and *Anopheles hermsi*, provided a lack of clarity. However, the sequencing of the ITS2 locus was able to achieve our goal and the results indicate that the previously identified ranges of these two species may have been incorrect or have changed over time. Our data highlights the lack of knowledge revolving around these cryptic mosquitoes and the need to decipher the biological differences between these understudied vectors.

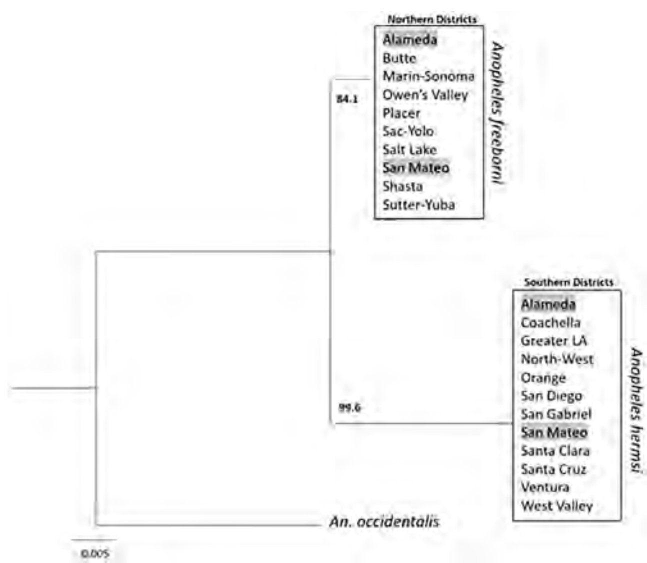

Figure 1.—Concatenated maximum-likelihood tree of Anopheles ITS2 sequences. Analysis based on a 450 base pair sequence of the locus. The two species largely grouped as expected, with *Anopheles freeborni* grouped with the Northern Districts and *Anopheles hermsi* with the Southern Districts, except Alameda and San Mateo which are highlighted in grey. These were unexpected locations where both species were found. Bootstrap values 70 and higher are shown. Outgroup (*Anopheles occidentalis*) is included.

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An Update on Research and Intern Projects at the Salt Lake City Mosquito Abatement District

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The Salt Lake City Mosquito Abatement District (SLCMAD) celebrated 100 years of operation in 2024. One of the key values of SLCMAD has been to innovate and improve mosquito surveillance and control through research (Rehbein 2024). Over the past three years SLCMAD has increased research activities through hiring two more full time staff members to focus on surveillance and research, increasing collaborations with universities and expanding the number of seasonal intern staff that are employed. Examples of university collaborations and internship projects over the last three years are highlighted here.

SLCMAD along with many other mosquito control agencies across the US often conduct aerial ultra low volume (ULV) applications of adulticides to reduce mosquito numbers and to interrupt virus transmission. An adulticide commonly used in these applications is Dibrom, which contains the active ingredient of Naled, an organophosphate. This active ingredient is approved for use by the US Environmental Protection Agency (EPA), recommended by the Centers for Disease Control and Prevention (CDC) and has been used for over 60 years in the US. Because of recent public interest in the safety of this pesticide, SLCMAD collaborated with an Atmospheric Scientist from the University of Utah with experience investigating air pollutants as well as researchers with expertise in pesticide risk and applications. This group worked to conduct a review of published literature on Naled and its major degradate, dichlorvous (DDVP), on human health. The review found that no studies provided evidence of a risk to human health when Naled is applied for mosquito control (Mendoza 2023).

To hire more interns to conduct research, SLCMAD worked with programs at the University of Utah (U of U) and the Entomological Society of America (ESA). District staff learned about intern programs sponsored at the U of U called Community Engaged Learning and Science Research Initiative (https://science.utah.edu/sri/). These interns often started as part time positions, but some of the students later transitioned to full time internships during the summer. SLCMAD also applied for the Public Health Entomology for All (PHEFA) program that was sponsored by the ESA and CDC (https://www.entsoc.org/advocacy-initiatives/public-health-entomology-for-all). Through this program our District was able to have two students from a local community college work full time for the summer.

One of the projects that the two interns worked on, was investigating potential toxicants for attractive toxic sugar baits (ATSB). Previously, SLCMAD staff researched the potential use of sugar alcohols in ATSBs, whereas these interns investigated other potential toxicants, including propylene glycol and the essential oils - garlic oil, cedarwood oil, papaya seed oil, cinnamon leaf oil, clove stem oil and patchouli oil. Results showed propylene glycol may be a good supporting ingredient in ATSB formulas and papaya seed oil showed promising mortality in laboratory trials (Maes 2024).

Another intern investigated previous erythritol toxicity findings to try and determine the mechanism(s) by which the sugar alcohol leads to mortality in mosquitoes. Results of the studies showed that erythritol is a competitive inhibitor of α-glucosidase, a diuretic in adult mosquitoes (Nelson 2024). The effects were not likely to be acute or toxic enough on their own, but show erythritol may work in combination with other toxicants in a bait formulation.

Sugar feeding by mosquitoes on flowers found in the Salt Lake area was studied by another intern. The purpose of this research was to determine which flowers are most attractive to sugar feeding mosquitoes at different times of the year, with the goal of using this information to best use ATSBs in the future. Mosquitoes were found to have different preferences for various flowering plants.

The oviposition behavior of the Western Treehole mosquito, *Aedes sierrensis,* was a topic of research as well. An intern determined that one of the attractive qualities of an oviposition site to *Ae. sierrensis* is color. This mosquito was attracted to purple when the water or the container of a potential oviposition site dyed this color (Bibbs 2024). Information from these studies may be used to improve surveillance for *Ae. sierrensis* in the future.

Another intern at SLCMAD investigated the presence of *Dirofilaria immitis*, the parasitic worm that causes dog heartworm, at the Hogle Zoo. The investigation was conducted by setting CO_2 baited traps at 5 different locations throughout the zoo on a weekly basis. Mosquitoes were identified and enumerated. Pools of up to 20 mosquitoes were tested for *Dirofilaria immitis* using a previously developed SYBR green assay (Spence Baeulieu 2020). In the summer of 2023 two of the 214 mosquito pools tested were positive for the infectious worm.

In the mid-July 2022 a new state correctional facility opened in a rural wetland habitat of SLC. One of the insect pests that workers at the correctional facility complained about in addition to the numerous mosquitoes was biting flies. In 2023 an intern evaluated two different types of biting fly traps near the prison to see which one was most effective in this habitat. The two traps used in the study were the Nzi Trap and the H-Trap. Results of the evaluation showed that the H-Trap on average collected more biting flies. The H-trap was also easier to set up and had less operational problems through the course of the summer.

One last project worked on was a collaboration with the nonprofit organization, The Ouelessebougou Alliance (OA), the University of Bamako, and the Anastasia Mosquito Control District (https://amcdsjc.org/). OA has provided humanitarian work in the Ouelessebougou region of Mali Africa for over 35 years (https://www.lifteachother.org/). One recent project they initiated was to provide relief from malaria through a 'US-style' of mosquito control. In 2023 a worker from Mali came to the Anastasia Mosquito Control District and SLCMAD for training. Then staff from these two Districts travelled to Mali to help set up a mosquito control program and provide additional training. This project focused on finding and treating larval sources to reduce mosquito populations and to conduct routine ULV applications by truck, preceding and during the peak malaria transmission season to reduce the parasite-infected mosquito population. Effectiveness is being determined by comparing the abundance and infection rates in mosquitoes at villages that were treated and those that were not treated. The mosquito control work was well received by the villages where it was initiated and it is planned to continue.

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Response to local transmission of dengue: Panel discussion summary

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Introduction

The dengue symposium at the 92nd Annual Conference of the Mosquito and Vector Control Association of California (MVCAC) detailed the first detections of local dengue virus transmission in the state of California. In the last decade, *Aedes aegypti* and *Aedes albopictus* have moved from initial detections to repeated collections throughout the state. Whether through repeated introductions or niche habitat utilization, these species now are routinely identified in urbanized environments near human dwellings. Meanwhile, areas of the world where dengue virus is considered endemic are identifying increasing trends of human detections, severe dengue cases and fatality rates. As these trends rise and globalized travel re-establishes in the wake of the COVID-19 pandemic, the conditions for local exotic pathogen transmission materialize. With symptomatic and asymptomatic cases originating from these pathogen rich areas and a competent vector routinely identified within densely populated areas, localized transmission was an inevitability. This symposium explored both cases of locally transmitted dengue virus in Pasadena and Long Beach, CA from the perspectives of the state health department, local health officials and local vector control. At the culmination of the presentations a brief question and answer panel discussion was held to explore the details of the experience.

Panelist presentations

To begin the symposium, Andrea Lund, an Epidemiologist and Disease Ecologist with the California Department of Public Health (CDPH), provided technical information regarding dengue virus's status in California and the recommended state guidelines for managing an exotic *Aedes*-borne pathogen. Dengue virus is considered a non-endemic pathogen in California, with routine introductions by travelers from endemic tropical and subtropical areas where *Aedes* mosquitoes thrive. The primary vectors for dengue virus are *Aedes aegypti* and the *Aedes albopictus* which are not native to California but invaded multiple counties. CDPH was alerted to the Pasadena and Long Beach cases by local health departments and provided recommended actions to health department and vector control partners. These response actions were outlined in the "Guidance for Surveillance of and Response to Invasive *Aedes* Mosquitoes and Dengue, Chikungunya, and Zika in California". Some key takeaways from the recommendations included maintaining regular communication between the local health department and the responding vector control agency, establishing a 45-day control period of potential transmission around the identified case, and continuing human and mosquito pathogen testing in the area of concern.

Discussing the first case of local dengue transmission, Matthew Feaster, the Division Manager with the Pasadena Public Health Department (PPHD), outlined the actions of the health department regarding the Pasadena case. The PPHD was alerted by a local hospital to a potential human case of dengue virus with no travel history. While PPHD began steps for confirmatory testing, they simultaneously contacted the local vector control agency, San Gabriel Valley Mosquito and Vector Control District, to coordinate their respective response. Once additional testing confirmed the patient's status of dengue virus infection without history of travel, an emergency press conference was held to issue statements from the city, health department and vector control agency about the severity of the situation, the lack of precedence for the diagnosis, and the actions moving forward. With official statements released, PPHD moved to local interventions involving neighborhood canvassing, resident interviews and when agreed upon, resident sera collections for additional human surveillance in the target neighborhood. These sera collections resulted in a second confirmed dengue infection in the immediate area with no additional travel history.

Finalizing the Pasadena case's presentations, Jason Farned, the District Manager of the San Gabriel Valley Mosquito and Vector Control District (SGVMVCD), depicted the actions vector control underwent in response to these cases of local transmission. SGVMVCD was informed of the suspect case by PPHD and worked in cooperation throughout the incident. A treatment area was established according to PPHD's case information, initiating the 45-day window of control recommended by the CDPH for *Aedes*-borne transmission and allowing for preliminary surveillance/door-to-door property inspections to take place. This preliminary surveillance and treatment indicated a need for additional larviciding and adulticiding which took place the following week. Surveillance and control continued in the immediate area when confirmatory tests verified the accuracy of a dengue positive human case with no travel history. With this confirmation and a completed press conference announcing this case to the public, a second application of adulticiding and larviciding was executed in the target area due to repeated elevated abundance of *Aedes aegypti*. Additional mosquito testing, property canvassing, and education continued in the target area until the 45-day recommended window expired and vector control concluded their activities for the case.

Closing out the presentation portion of the symposium, Judeth Lagrimas Luong, Manager of the Environmental Health Bureau with the City of Long Beach Department of Health and Human Services (LBHHS), discussed the second local dengue transmission with a brief interlude from Steve Vetrone, the Director of Scientific Technical Services at the Greater Los Angeles County Vector Control District (GLACVCD), who discussed what aid the vector control agency was able to provide. Similar to the Pasadena case, LBHHS was alerted to a potential dengue case without travel history and made plans for confirmatory testing. An aspect of this confirmatory testing concluded that the Long Beach and Pasadena cases were not related and originated from separate dengue virus introductions. Once confirmatory testing was completed and indicated a positive dengue case with no travel history, LBHHS vector division partnered with GLACVCD to perform adulticiding applications and door-to-door inspections around the target area. No additional cases were identified and with low vector abundance after the aforementioned treatment were conducted, control operations were concluded.

Concluding panelist discussion

With the presentations concluded, each speaker was invited to discuss as a group the lessons learned from each of these cases and the perspectives gained at a state, health department and vector control agency level directed by audience questions. Although the discussion extended well beyond the scheduled time limit for the symposium, audience members continued to be engaged in the conversation and displayed an interest in this topic potentially being discussed at future Annual Conferences. The questions focused on a variety of topics from public engagement, factors that may have led to these cases occurring and the inclusion of the CDC and other health organizations among other topics. Significant among the conversation though was the emphasis on multi-organization collaborations, the pre-emptive use of resources and the willingness to share resources in times of need. In both cases of local transmission, several groups acted simultaneously to gather, share, or utilize resources in an expedited manner. Having these channels of communication be practiced and known allowed for an effective precedent to be set when mitigating an exotic local disease transmission.

Implementation of Unmanned Aircraft System (UAS) into a Vector Control Program

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Introduction

Unmanned aerial equipment has been a topic of discussion in many mosquito control districts. Fully committing to an Unmanned Aircraft System (UAS) requires licensing and training for the agency and especially the department users. In 2013, Leading Edge Aerial Technology demonstrated a remotely controlled aircraft equipped with a liquid application system to the Coachella Valley Mosquito and Vector Control District (CVMVCD) (Figure 1). The person manipulating the Ground Control Station controlled the aircraft and the application system simultaneously. This demonstration served as our introduction to a UAS platform. The technology associated with this and other UAS was still evolving, and therefore developing a program was still unknown technology. As a result, CVMVCD has continued to explore alternative aerial application equipment in lieu of manned aircraft.

Equipment acquisition

In 2017, the decision was made to acquire two small UASs, the Matrice 600 Pro (Figure 2) and the Phantom 4 Pro (Figure 3), both from Da-Jiang Innovations (DJI, Sky City, Nanshan District, Shenzhen, China), a leader in drone technology. These models were chosen for their imagery capabilities, and the payload carrying ability for the Matrice 600 Pro which could be fitted with an aerial application system. Onboarding these unmanned aircraft systems at CVMVCD included legal requirements, 14 CFR Part 107 and 14 CFR Part 47 or Part 48 from the Federal Aviation Administration (FAA).

After fulfilling all FAA requirements and receiving both small UAS systems in 2018, CVMVCD was capable of capturing aerial images of mosquito larval habitats (Figure 4). As we progressed with our small UAS flights, the sheer quantity of imagery that was captured became daunting. To keep track of the location of each image, mapping software was required for better organization. Our Information

Figure 1.—Leading Edge Aerial Technology remote controlled aircraft equipped with a liquid application system.

Figure 2.—DJI Matrice 600 Pro imagery small UAS.

Technology (IT) department suggested a Map Pilot Pro software application from Maps Made Easy (www.mapsmadeeasy.com) that could capture this information, making our UAS mapping more efficient and productive. During our small UAS flights, we utilized this mapping application to apply flight data to make informed abatement decisions (Figure 5).

In 2022, CVMVCD acquired an application UAS (Precision Vision 35X) for mosquito control applications which required additional FAA and CDPR licensing. After meeting all the necessary certifications and licensing requirements, our focus again shifted towards deploying this unit to the field as soon as possible. Loading and transportation was split between two trucks (Figure 6).

Problems encountered and their solution

To ensure the safe and successful completion of each UAS flight mission several factors were addressed, including wind speed and direction, pounds per acre, product size, air temperature, distance and Visual Line of Site (VLOS). An important factor for our flight missions

Figure 3.—DJI Phantom 4 Pro imagery small UAS.

Figure 4—●●●.

was ensuring that we had sufficient batteries. The hot desert temperature increased power consumption and the heat generated during each flight required a cool down period before recharging. This was facilitated by cooling the batteries with our truck air conditioning vents (Figure 7). This reduced the overall recharge time and became our normal practice when operating at elevated temperatures.

Further support was provided by an enclosed trailer that contained a split air conditioning system and a 30-amp power distribution center with multiple 120-volt power outlets, dedicated battery charging area and storage cabinets (Figure 8, 9). Power supply provided by a Yamaha Inverter generator model EF6300iSDE.

Conclusions

The introduction of small UAS systems paved the way for the acquisition of the application Precision Vision 35X. Although there may be differences in size and maneuverability, the basic physical components of the aircraft are uniform across models. This includes blades, motors, battery, ground control station, and pilot. Some UAS system platforms may have more powerful motors or be better equipped for certain tasks, but the underlying technology remains consistent.

The UAS program in the Coachella Valley has been impacted by the intense heat that can cause electronic devices to work harder and result in slower response times. This can

Figure 5.—UAS Map Pilot Pro mapping application displaying flight data.

Figure 6.—UAS (Precision Vision 35X) operations at duck clubs near the Salton Sea.

lead to issues such as app crashes and unresponsive devices. To address this, we have implemented a Mobile UAS workstation capable of cooling all electronic devices and transporting equipment for our UAS program. Although these steps may seem simple, they are crucial for the success of our UAS missions in the Coachella Valley. This UAS mobile workstation would serve all UAS current and future needs of one team, with room to grow while focusing on our mission to protect public health and control mosquito populations.

Figure 7.—DJI Phantom 4 Pro UAS battery recharging in the truck cab with air conditioning cooling the batteries.

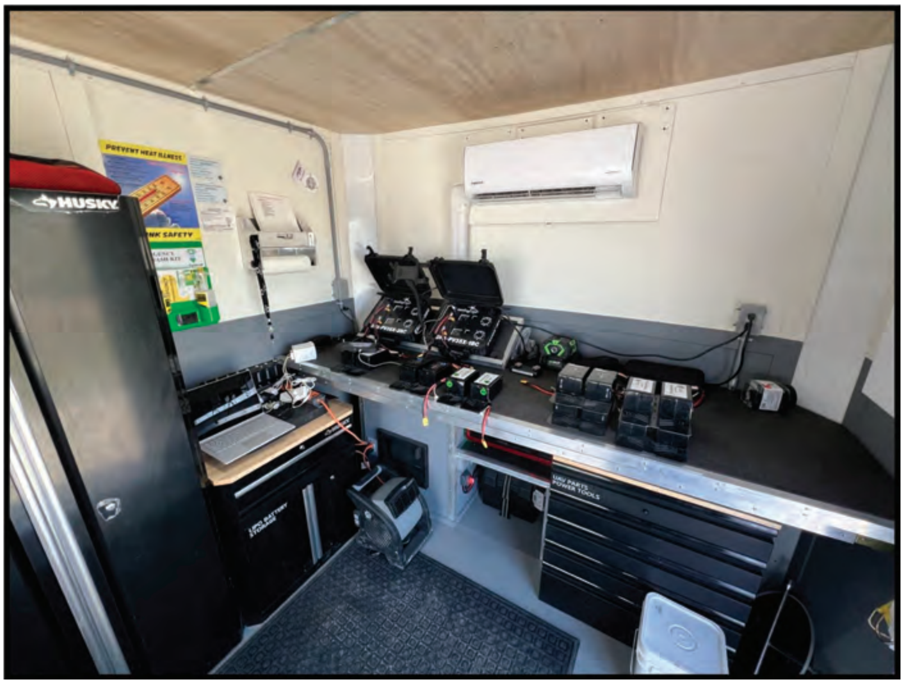

Figure 8.—Interior of the support trailer with charging station, work space and storage cabinets.

Figure 9.—Enclosed 22 foot long support trailer.

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Edward Prendez, Information Technology Manager: Vincent Valenzula, Lead Technician; Gregorio Alvarado Operations Manager; Michael Martinez, Field Supervisor; Oldembour Avalos, Field Supervisor; Manager; Kim Y. Hung, PhD, Vector Ecologist; Jennifer A. Henke, MS, Laboratory Manager: Ryan Gonzales, Technician II: Jonathan Zamaniego, Technician I: Iver Romero, Technician I.

Utilizing drones in Contra Costa County to conduct larviciding treatments during an above average rainfall mosquito season

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Abstract

During the first four months of 2023, Contra Costa County experienced above average rainfall leading the Contra Costa Mosquito and Vector Control District (District) to consider alternative methods to effectively control sites throughout the county that were producing mosquito larva. Previous methods included utilizing a helicopter, but this method was not viable due to certain sites size, location, and/or limitations due to proximity to homes or protected areas; however, District employees had no previous experience with drone applications. As this presentation showed, the challenges brought on by the above average rainfall became a learning opportunity which revealed the potential of integrating drones into the District's integrated vector management program as a tool for mosquito control.

UAS Real-Time wind speed data collection

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Introduction

The Sacramento-Yolo Mosquito and Vector Control District (District) (SYMVCD) has operated a small Unmanned Aircraft System (sUAS) program since early 2016. Besides aerial imaging, modeling, surveying, and mosquito control applications, the District also has utilized the sUAS onboard wind metering data as part of the operational decision making process. Previously, the District experimented with monitoring temperature, humidity and wind speed at different elevations above ground level (AGL) relative to time of sunset during aerial applications. Although this information has helped to determine wind trends in certain areas of the District under different environmental conditions, it has not been used for other operational applications.

Wind speed is critical for optimal Ultra Low Volume (ULV) mosquito control operations. Although mosquito control ULV operations often rely on basic wind reading measurements prior to product application, product efficacy trials require proper product calibrations and accurate wind and temperature readings. Ideal wind speeds for product application are usually between four and five mph with a maximum of 10 mph, and are necessary to ensure optimal product drift over targeted sites. Several types of wind collection devices exist on the market; however, the handheld Kestrel style wind meters (Neilsen-Kellerman Co, Boothwyn, PA, USA) tend to be the industry standard. These must be mounted on poles and tripods at around 30 feet AGL and at application release height to obtain true temperature inversion measurements (Armed Forces Pest

Figure 1.—Image of trial transect grid with the intended driving location.

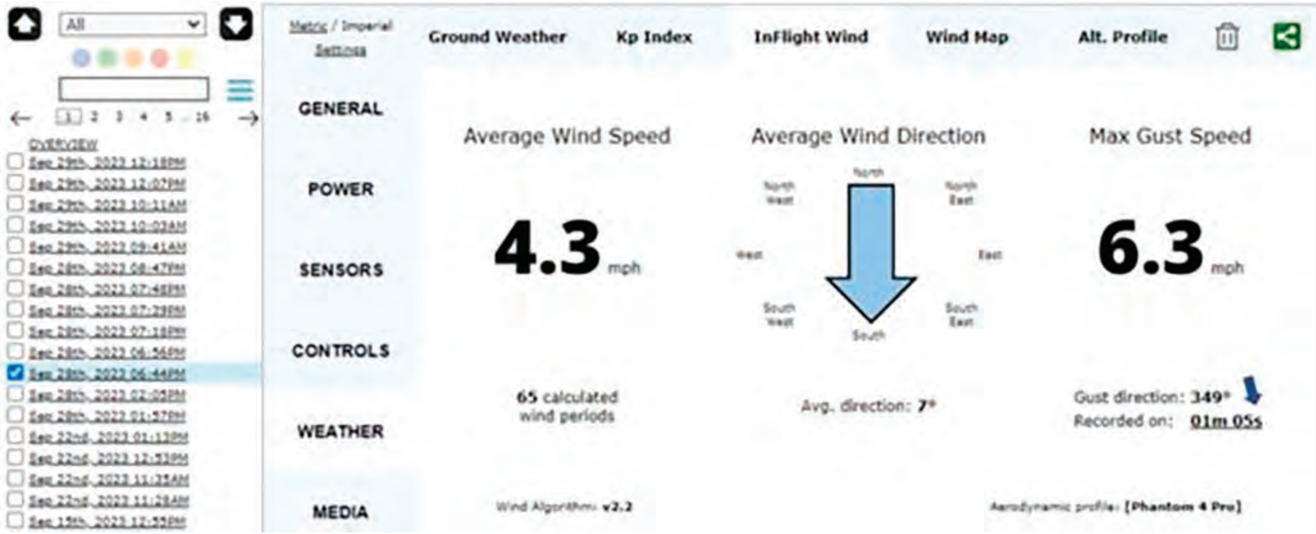

Figure 2.—Initial wind speed captured by the UAS prior to trial spray as visualized in Airdata.com.

Management Board Technical Guide No. 13, March 2019). Utilizing off the shelve sUAS technologies may be an additional tool to accurately collect and analyze wind speed and direction as well as temperature data at the time of ULV applications.

Methods

The District owns several sUAS aircraft DJI Phantom models (DJI, Nanshan, Shenzhen, PRC). These aircraft utilize a series of internal proprietary algorithms to control the propellers while in flight to maintain the desired elevation and position even when wind gusts are present. The algorithms help compose downloadable flight data, which includes wind speed, with extreme accuracy. The District aims to utilize this feature from the sUAS program to help guide future operational decisions, particularly, when to begin ULV applications during field trials. For this assessment, we employed the sUAS alongside handheld Kestrel wind meters to monitor and compare wind speeds before and during the trial.

During normal operation, the sUAS is taken up to 30 feet AGL and allowed to hover from a few seconds to a couple of minutes. Upon landing, flight data is synced from the sUAS manufacturer's flight app (DJI Go4 app, DJI, Nanshan, Shenzhen, PRC) to the AirData UAV proprietary software called HDSync (AirData UAV, El Dorado Hills, CA, USA) which allows for viewing and data downloading from the AirData UAV website (www.airdatauav.com). This process takes approximately 30 sec after landing, depending on local internet speed. This technology is compatible with the majority of commercially available sUAS platforms. Before nighttime flights, the SYMVCD ensured that all applicable local and federal sUAS licensing, waivers, and aircraft lighting regulations were meet.

ULV trial

The ULV trial was set up in an open field south of Elk Grove (CA, USA) that allowed access to multiple sides of the field during the trial. Due to average and expected wind directions, the trial was set up so the ULV trucks, equipped with a London Fogger, would drive on the western side of the field. Three rows of three stations were set in a grid 100 feet apart and starting 100 feet away from the truck to capture a full 300 feet swath width (Figure 1). The products used for this trial were DeltaGard (Deltamethrin 2% AI) (Bayer Environmental Science, Research Triangle Park, NC) and malathion (Fyfanon EW®, FMC Corporation, Philadelphia, PA, USA).

Figure 3.—Compass depicting degrees using for reporting wind direction.

	Flight time	Altitude	Home Distance	Wind Direction	Wind Speed
A	00m 25s	27.9 ft	34 ft	257°	3.33 mph
B	00m 30s	27.6 ft	34 ft	276°	4.21 mph
C	00m 35s	27.6 ft	35 ft	268°	7.38 mph
D	00m 40s	27.9 ft	35 ft	270°	7.40 mph
E	00m 45s	27.9 ft	35 ft	278°	7.81 mph
F	01m 05s	38.4 ft	63 ft	271°	6.46 mph
G	01m 15s	38.4 ft	71 ft	274°	7.29 mph
H	01m 20s	38.4 ft	71 ft	268°	11.01 mph
I	01m 25s	38.4 ft	70 ft	268°	10.76 mph

Figure 4.—Map within Airdata.com showing wind speeds and directions at all locations flown during that mission.

Each station consisted of a vane holding five cages of different adult mosquito populations and one tripod with a set of spinning slides to capture product droplet deposition. A non-treatment control was set on the South corner of the trial grid that contained mosquito control cages, control spinners, and two Kestrel wind meters, one at five feet, the application delivery height, and one on a 30 foot high extended pole, which is the temperature inversion height, with both devices mounted to a self directional vane that could follow wind direction. The expectation was that the post sunset winds would be coming from the Southwest, with the application delivery truck to be driven from the Southwest and follow an existing roadway along the western side of the trial, drifting the product across the trial grid and reach every station. The application started 30 min post sunset. An internet enabled smartphone or tablet with the sUAS software was used to view and download the data.

To capture wind readings, the sUAS was flown before sunset and near the control locations on the Southwest corner of the field while the ULV trial was being set up.

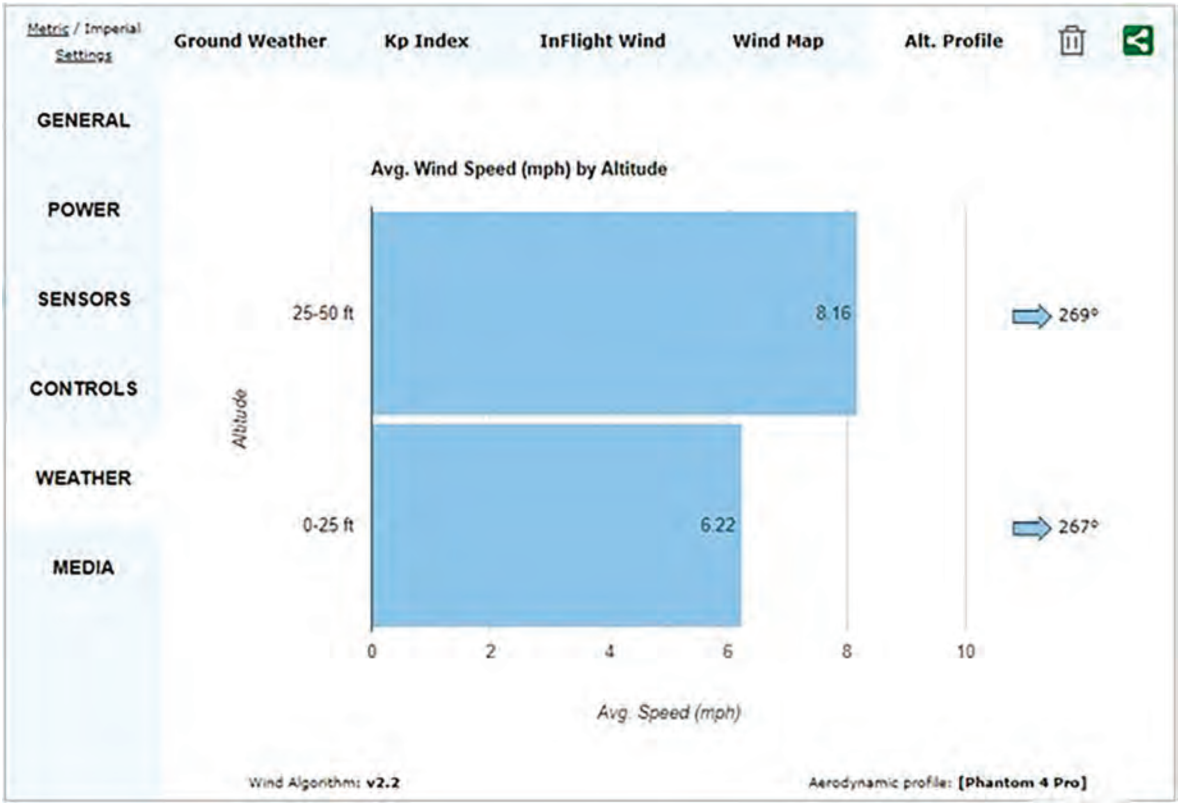

Figure 5.—Alternative display of wind data from Airdata.com in graph form.

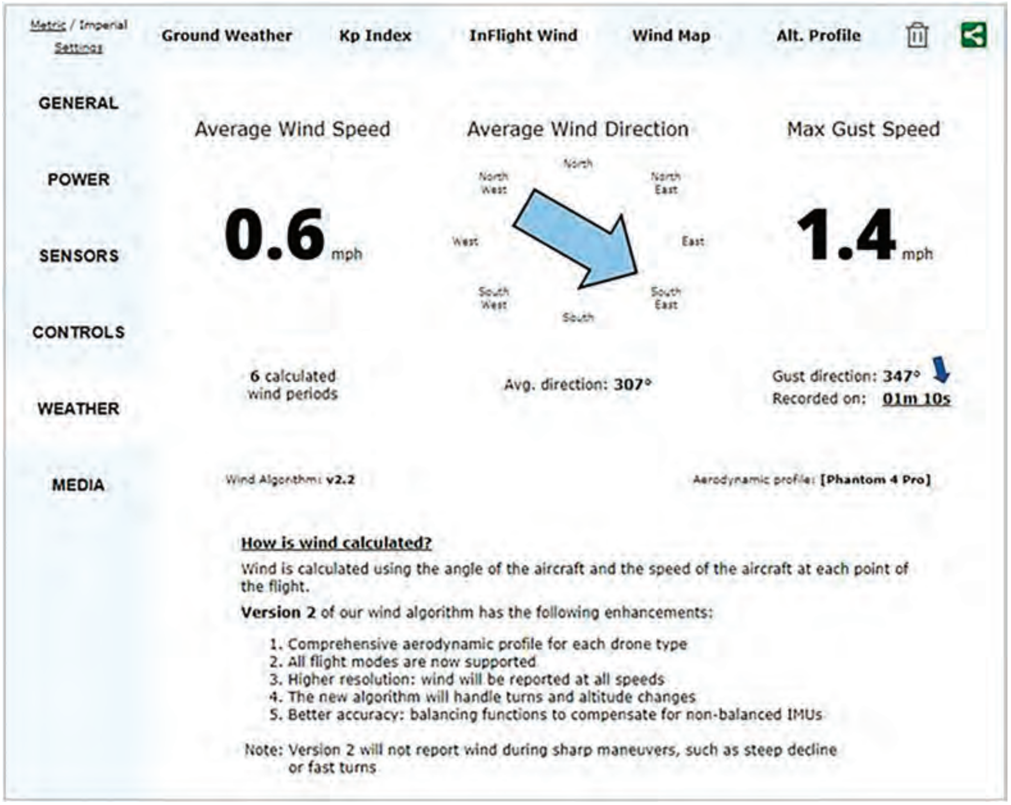

Figure 6.—Data from Airdata.com showing varying wind speeds and direction towards Southwest at sunset prior to the start of the trial.

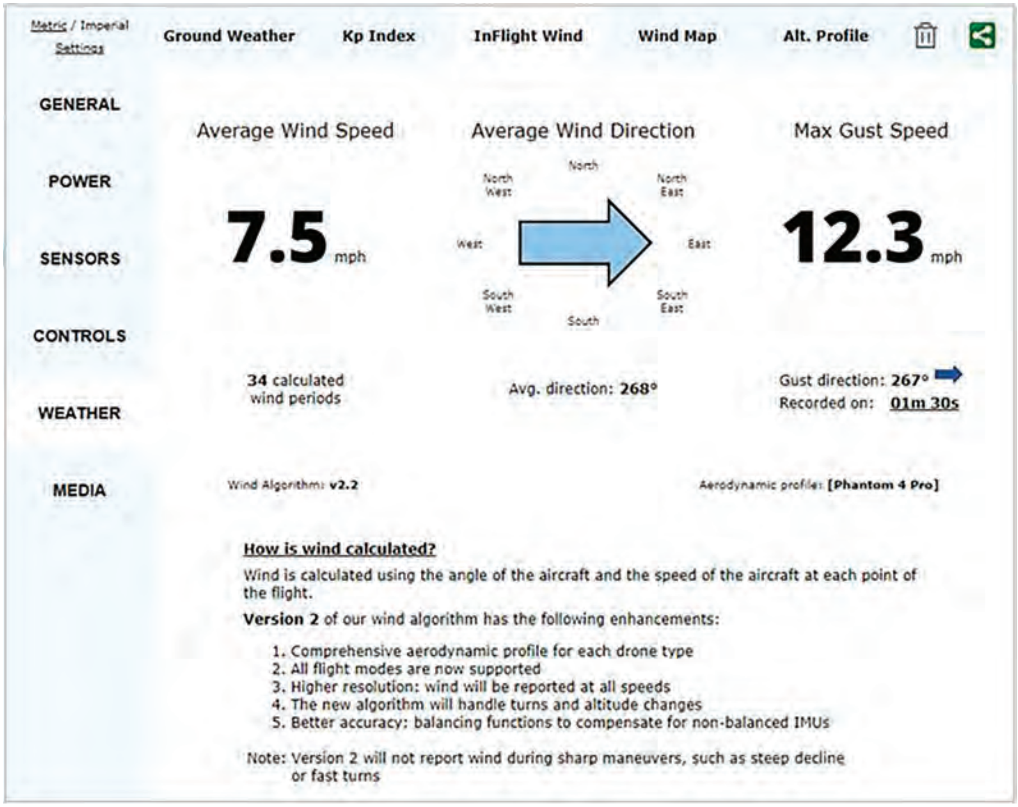

Figure 7.—Data from Airdata.com showing varying wind speeds and direction towards the West after sunset.

Wind readings were taken post sunset, and then every 30 min until a desirable wind speed was detected to conduct the trial. Each flight was up to 30 feet AGL and hovered for approximately three min before landing. Upon landing, the flight data was synchronized using the DJI Go4 app. When the fight record finished uploading, the flight data was synced using the HDSync software. All flight data, including wind speed information, was available for viewing on the AirData sUAV's website (www.airdatauav.com) via a paid annual subscription. This entire process took approximately 30 sec.

Results and Discussion

Initial wind speed readings before the trial showed that the wind was coming from the North, directly opposite of the needed direction for correct product application and drifting. As seen in Figure *2*, the AirData UAV platform provided very detailed flight data including wind speed, which was viewable in multiple formats (Figures 2, 4, and 5). Wind direction was displayed in directional degrees based upon magnetic North (Figure 3).

After 45 min post sunset, the wind direction began to change and started shifting towards the West (Figure 6) and at 1:53 hrs post sunset the wind finally shifted and steadied at due West. While the sUAS readings showed that the wind was coming from due West (direction 268°) with an average wind speed of 7.5 mph, the 30 foot mounted kestrel wind reader only showed an average speed of 5.4 mph with a Westerly direction (233°) (Figure 7). Both devices showed wind speeds within the optimal ULV application conditions, signaling that the application could start as scheduled. It is worth noting that the sUAS readings showed that the wind speed varied, and that very little wind speed existed post sunset until the start of the trial (Figures 6 and 7).

Conclusion

Although the overall sUAS wind speed and directional data was difficult to verify completely due to field level inaccuracies (as opposed to wind tunnel testing), the use of handheld Kestrel wind meter comparisons indicated that the sUAS meteorological data could be utilized in mosquito control operational decision making. Additionally, the sUAS can collect data from multiple locations as they can be flown into and above the product application block up to four hundred feet AGL, thus providing more accurate location-based data. The use of sUAS platforms for meteorological data collection is also a value-added benefit for mosquito control programs that already own or are planning to deploy sUAS.

Acknowledgement

Sarah Wheeler coordinated the trial and Ruben Rosas was an additional UAS Pilot during trial.