MVCAC

Proceedings and Papers

OF THE EIGHTY-SEVENTH ANNUAL CONFERENCE OF THE Mosquito and Vector Control Association of California

October, 2020

ISSN 1554-4974 Volume 88 No.1

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PROCEEDINGS AND PAPERS

OF THE

Eighty-Eighth Annual Conference of the Mosquito and Vector Control Association of California

January 26 – 28, 2020
Held at the Hyatt Regency Mission Bay
San Diego, California

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Published October 2020

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Symposium: Mosquito Operations and Technology

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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
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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 behavior of *Culex tarsalis*

Quantifying sociodemographic heterogeneities in the distribution of *Aedes aegypti* among California households*

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*Results of this study have been submitted to PLOS Neglected Tropical Diseases (Donnelly et al. In review).

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Introduction

The spread of *Aedes aegypti* in California and other regions of the U.S. has increased the need to understand the potential for local *Ae. aegypti*-borne pathogen transmission, particularly in arid regions where the ecology of these mosquitoes is less understood. *Ae. aegypti* is the principal urban vector of Zika virus (ZIKV), dengue virus (DENV), and chikungunya virus (CHIKV) (Halstead 1990, Vega-Rúa et al. 2014, Petersen et al. 2016) and has adapted to thrive in urban environments. *Ae. aegypti* feed frequently and primarily on humans, which makes *Ae. aegypti* a uniquely effective and important vector for arboviral transmission between humans (Scott et al. 1993, Richards et al. 2006, Barrera et al. 2012, Bowman et al. 2016). Moreover, *Ae. aegypti* tend to cluster at very fine spatial scales, only dispersing short distances up to a few hundred meters, suggesting that ZIKV, DENV, and CHIKV risk also varies at the scale of the household (Getis et al. 2003, Harrington et al. 2005, Maciel-de-Freitas et al. 2010). Although the primary public-health intervention to prevent arboviral outbreaks is reducing human-mosquito contact through vector control and personal protective measures, there is a need to develop methods that would minimize human-*Ae. aegypti* contact in the arid Southwest, where climatic and sociodemographic factors differ from those in the tropics where DENV is endemic. For public health and vector control programs, it is also helpful to know whether variation in risk can be attributed to sociodemographic factors that could help to target surveillance and control programs. Sociodemographic factors have been shown to influence transmission risk of dengue outside the U.S. by modifying biting rates and vector abundance. A number of previous studies have investigated the urban characteristics associated with DENV transmission and *Ae. aegypti* ecology. Poor housing conditions, high population and housing densities, and low socioeconomic status (SES) have been associated with dengue in the tropics (Waterman et al. 1985, Caprara et al. 2009, Kikuti et al. 2015, Mulligan et al. 2015). Similarly in the U.S., specific household characteristics and human behaviors that modify human-mosquito contact have been associated with DENV; in two studies conducted on the Texas-Mexico border, lack of air-conditioner usage was associated with higher DENV seroprevalence (Reiter et al. 2003, Ramos et al. 2008). In regions of the U.S. where *Ae. aegypti* recently have invaded and where residential areas are structured much differently than those in regions of the world where *Ae. aegypti* are endemic (i.e. the tropics), it is unclear how sociodemographic factors may modify the spread and abundance of *Ae. aegypti* populations and translate to the risk of human-mosquito contact.

Aedes aegypti were first detected in California in 2013 and have since spread throughout the Central Valley and Southern California. In Los Angeles County in Southern California, controlling *Ae. aegypti* populations has been particularly difficult, and eradication now seems unattainable. Since the first detection in Los Angeles County in 2014, *Ae. aegypti* has spread to over 80 cities within the county as of November 2019. Vector abundance is an important and highly variable determinant of vectorial capacity. Understanding *Ae. aegypti* abundance heterogeneity among households is essential for understanding the risk of local ZIKV, DENV, and CHIKV transmission in California and other areas where these viruses could cause local outbreaks. We conducted a cross-sectional study in Los Angeles County during summer 2017 to understand the causes of variation in relative abundance of *Ae. aegypti* among households.

Methods

We surveyed 161 houses across six sociodemographically diverse cities in LA: Boyle Heights, Commerce, East Los Angeles, Downey, La Mirada, and Whittier. Sampled households were located in census tracts with median household incomes ranging from $21,299- $136,793. These six cities present a diverse range of incomes, and all had similar dates of initial *Ae. aegypti* detection between August 2015 and August 2016. Surveys consisted of systematic adult mosquito collections, inspections of households and surrounding properties, and administration of a questionnaire in English or Spanish. Households were surveyed for human behavioral and household characteristics, including those that may affect *Ae. aegypti* biting exposure and breeding. We obtained sociodemographic variables, including median household income, population density, and property size and used generalized linear models to determine whether behavioral, household, and socioeconomic factors were associated with increased adult *Ae. aegypti* abundance.

Results and Discussion

We surveyed 161 households in Los Angeles County. Adult *Ae. aegypti* were detected at 72% of households and were found indoors at 14.4% of households. An average of 3.1 *Ae. aegypti* were collected per household. *Ae. aegypti* abundance outdoors was higher in lower-income neighborhoods and around older households with larger outdoor areas, greater densities of containers with standing water, less frequent yard maintenance, and greater air-conditioning usage. We also found that *Ae. aegypti* abundance indoors was higher in houses that had less window and door screening, less air-conditioner usage, more potted plants indoors, more rain-exposed containers around the home, and lower neighborhood human population densities. These results suggest that, in California, there are behavioral and sociodemographic determinants of *Ae. aegypti* abundance, and that low income, Los Angeles households with the characteristics described in this study could be at higher risk for exposure to *Ae. aegypti* biting and potentially greater risk for Zika, dengue, and chikungunya virus transmission if a local outbreak were to occur.

Acknowledgements

We thank the staff of the Greater Los Angeles County Vector Control District for hosting our field work and providing guidance and support during field collections. We also thank the undergraduate students who helped to collect data in Los Angeles and aided in laboratory work: Tiffany Tan (University of California, Irvine), Khin KyiSin (University of California, Los Angeles), Chelsea Galicia (Whittier College), Claire Chapman (University of California, Davis). Financial support for this study was provided by National Aeronautics and Space Administration's Applied Sciences Program in Health and Air Quality (Grant NNX15AF36G) and startup funds to CMB from the School of Veterinary Medicine, University of California, Davis. CMB also acknowledges support from and the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement U01CK000516).

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Semiochemicals associated with the western mosquitofish, *Gambusia affinis*, and their effect on the oviposition behavior of *Culex tarsalis**

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*A portion of this manuscript was published previously in the Journal of Medical Entomology (Why and Walton 2019).

INTRODUCTION

Many studies have shown that aquatic organisms use chemical cues for a variety of purposes including locating food, reproduction and mate finding, navigation, long-distance migration, and detection of predators (Brönmark and Hansson 2012). Semiochemicals are defined as chemicals that carry information and mediate an interaction between two individuals. Either the sender, receiver, or both organisms benefit from the release of the semiochemical (Brönmark and Hansson 2012). Chemical cues also may provide a wealth of information about the sender, such as sex, social status, diet and species identity. Cues also may indicate the presence of a predator (Brönmark and Hansson 2012).

Semiochemicals may be particularly advantageous in aquatic systems, compared to terrestrial systems, because the use of visual cues can be significantly reduced due to attenuation of light with increasing depth and turbidity in the water column (Brönmark and Hansson 2012). Many aquatic insects have complex life cycles that involve multiple instars and variable growth periods in the nymphal/larval stages that depend on temperature and food fluctuations, thus leaving the immature stages vulnerable to prolonged periods of predation. The detection of predators by aquatic insects is not restricted to the immature stages; adults searching for oviposition sites also may respond to the presence of predator cues (Silberbush and Blaustein 2008; Silberbush et al. 2010). There is strong selection pressure on females of a given species to make accurate egg-laying decisions during oviposition (von Elert 2012). Gravid female mosquitoes use a combination of physical, biological, and chemical cues from the environment to select oviposition sites (Benzon and Apperson 1988; Bentley and Day 1989; Isoe and Millar 1995). *Culex tarsalis* Coquillett, the western encephalitis mosquito, frequently lays egg rafts in vegetation that has been inundated with water, such as rice fields, sumps, and wetlands (Bohart and Washino 1978) and these habitats often contain both fish and invertebrate predators.

The present study investigated the responses of female *Cx. tarsalis* to semiochemicals associated with the western mosquitofish, *Gambusia affinis* (Baird & Girard), in oviposition sites and addressed potential shortcomings of previous oviposition studies by investigating how chemicals associated with fish affect mosquito oviposition behavior. This study also characterized the semiochemicals associated with mosquitofish that influenced *Cx. tarsalis* oviposition.

METHODS

First, to determine if semiochemical concentration affected the ovipositional responses of female *Cx. tarsalis* in the laboratory, three ratios of fish:water (1 fish: 2 L; 1 fish: 1 L; 10 fish: 1 L) were used in binary choice assays, where aged tap water was used as the control. Single choice assays determined if females would oviposit in *Gambusia*-exudate water when alternative sites were not presented.

Second, binary choice assays tested different concentrations of cues that female *Cx. tarsalis* might encounter in nature using two concentrations (full-strength and diluted by 50% with pond water) of *Gambusia*-exudate pond water versus pond water alone. It is conceivable that factors other than fish-related semiochemicals (i.e., presence/absence of food for offspring or presence/absence of conspecifics) influence oviposition choices by female mosquitoes.

Third, oviposition site bioassays using multiple gravid females in a cage might be confounded by pheromones associated with *Culex* egg rafts. To remove this potential confounding effect, the oviposition responses of individual females were assessed in 55-ml plastic vials. Several hundred (N = 518) gravid mosquitoes were offered either fish-conditioned water or control water in vials that either retained volatile compounds or permitted volatile compounds to volatilize and escape.

Fourth, the composition of the blend of *Gambusia*-associated chemicals is unknown and may include volatile and non-volatile compounds. To force egg-laying mosquitoes to be in direct contact with a potential non-volatile stimulus at all times, trials with individual females (N = 97) that had part or all of their wings amputated were conducted.

Fifth, fish-associated semiochemicals have been hypothesized to originate from bacteria associated with the mucus on the surface of the fish (Forward and Rittschof 1999). However, the role of bacterial metabolic byproducts associated with fish that affect female mosquito oviposition behavior have not been well studied. To test whether bacterial metabolites contribute to the cues that deter egg-laying by mosquitoes (N = 90), *Gambusia*-exudate water was passed through membrane filters (pore size: 0.2 µm or 0.45 µm) to filter-sterilize the water and the resulting filtrate was tested in oviposition cups. We also compared unfiltered *Gambusia*-exudate water to filtered *Gambusia*-exudate water to see if there was an effect on mosquito oviposition.

Based on the results obtained in the oviposition choice trials, female *Cx. tarsalis* were considered to have reacted to volatile and non-volatile compounds. The objectives of this part of the study were to determine 1) what volatile compounds are present in the headspace of the *Gambusia*-treated water using solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC/MS), 2) how long the chemicals persist in the headspace after the fish have been removed from the water, 3) if any non-volatile chemical compounds are present in the *Gambusia*-treated water using a variety of analytical chemistry techniques, including solid-phase extraction (SPE), gas chromatography-mass spectrometry (GC/MS), GC/MS Time of Flight (GC/MS-TOF) and liquid chromatography-mass spectrometry (LC/MS), and 4) their effect on the oviposition behavior of female *Cx. tarsalis* using chemical standards of the identified compounds.

RESULTS AND DISCUSSION

Although female *Cx. tarsalis* were often deterred from laying eggs in oviposition sites that contained semiochemicals associated with *G. affinis*, this deterrence was not strongly associated with the density of fish used to condition the water in oviposition cups. There was approximately a 50% reduction in the number of egg rafts laid onto *Gambusia*-exudate water made with either aged tap water or pond water as compared to water controls that did not house mosquitofish.

Gravid mosquitoes tested individually to remove the potentially confounding effect of the presence of an egg pheromone on oviposition site choice did not reduce oviposition onto *Gambusia*-exudate water as compared to control water. Oviposition by clipped-wing female mosquitoes also did not differ significantly between *Gambusia*-exudate water and control treatments. The lack of differences between oviposition onto *Gambusia*-exudate water and control water might have been influenced by the inability of the gravid female mosquito to leave the oviposition site and acclimation/desensitization to chemicals deterring ovipostion during prolonged exposure.

Oviposition onto *Gambusia*-exudate water that had been filter-sterilized to remove the presence of bacteria was reduced relative to filter-sterilized control water, suggesting that semiochemicals were still present after bacteria were removed. Filter sterilization also reduced mosquito oviposition onto *Gambusia*-exudate water relative to unfiltered *Gambusia*-exudate water.

Three volatile compounds were identified in the headspace of *Gambusia*-exudate water: dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS) and S-methyl methanethiosulphonate. Differing concentrations of each volatile compound were tested in binary choice assays to determine their effects on *Cx. tarsalis* oviposition. Results from three types of bioassays (binary choice, no-choice bioassays, and clipped-wing bioassays) indicated that *Cx. tarsalis* females sometimes reduced oviposition when low concentrations of DTMS were present in oviposition water, but did not differentiate between oviposition cups containing aged tap water versus aged tap water containing either DMDS or S-methyl methanthiosulphonate at concentrations ranging from 0.01% to 0.001%.

In addition to these three volatile compounds, two non-volatile compounds were found in *Gambusia*-exudate water. *Culex tarsalis* females were significantly deterred from ovipositing onto water that contained the extract from *Gambusia*-exudate water when compared to aged tap water. Extracts were obtained by running *Gambusia*-exudate water through liquid chromatography columns to remove the chemicals present from the water molecules. The partial chemical structure of one of the compounds was characterized and was found to have a ring structure similar to that of cholesterol. Based on the results in these studies, it appears that when gravid *Cx. tarsalis* “taste” water containing these compounds, they elicit a deterrent behavioral response, leading to a decrease in oviposition.

CONCLUSIONS

Laboratory experiments indicated that female *Cx. tarsalis* can detect the presence of chemicals associated with the western mosquitofish, *G. affinis,* in oviposition sites leading to a decrease in oviposition. Of the three volatile compounds identified, only DMTS deterred ovipositing females in bioassays and only at particular concentrations. This lack of response to the volatile compounds may indicate that the female mosquito has found a suitable larval habitat, but it is only upon tasting the water and detecting the presence of the non-volatile compounds that she is then deterred from laying her eggs. Further elucidation of the non-volatile compounds present in *Gambusia*-exudate water needs to be carried out, and associated bioassays conducted, to determine if a new class of chemical compounds can be used for control of ovipositing mosquitoes.

Acknowledgements

We thank M. Wirth, D. Popko, C. Handayan, G. Martinez, D. Beasley, V. Chan and C. Umeda for help in the laboratory and field. Support was provided by the Bill and Melinda Gates Foundation, Robert and Peggy van den Bosch Memorial Scholarship, and the Jameson Foundation Scholarship. This work was supported, in part, by the USDA National Institute of Food and Agriculture, Hatch project 1007869 and the Agricultural Experiment Station at U.C. Riverside. W.E.W. acknowledges funding support during manuscript preparation from the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516). The vertebrate animals in these experiments were used in accordance with fair treatment of animals under protocols (chickens: A 20130030; fish: A 20130005) in compliance with the Institutional Animal Care and Use Committee. All experiments were carried out in compliance with the laws of the U.S.A., in which they were performed. This project was carried out in partial fulfillment of Ph.D. degree from the Department of Entomology, University of California, Riverside to A.M.W.

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Evaluating ivermectin-treated backyard chickens as a novel urban West Nile virus control strategy

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Introduction

Current vector control strategies rely primarily on pesticides to target mosquitoes involved in enzootic and zoonotic transmission of West Nile virus (WNV) (Rose 2001). Ivermectin (IVM), a widely used antiparasitic drug in human and veterinary medicine (Watts 2015, Laing et al. 2017), provides a potential alternative for targeted control of bird-feeding mosquitoes. Mosquitoes that ingest IVM experience increased mortality (Nguyen et al. 2019), so that few will survive long enough to transmit WNV during a future bloodmeal. Therefore, this strategy aims to prevent future bites and block subsequent transmission, reducing the overall local transmission of WNV. Our study, conducted in suburban neighborhoods across Davis, California, aimed to determine whether ivermectin delivered through backyard chicken flocks can suppress the abundance of WNV-infected mosquitoes and transmission of WNV as measured by chicken seroconversions.

Methods

We placed eight flocks with six chickens each in coops in backyards across Davis; four were treated with IVM-coated feed and four were untreated. Treatments were randomly allocated to flocks, and treated flocks received powdered IVM daily in their feed (200 mg IVM/kg feed) during routine husbandry, whereas untreated flocks received the same amount of feed without IVM. To quantify the effects on WNV, we monitored entomological indices using dry ice-baited traps (i.e., trap counts, WNV infection prevalence, and parity rates in *Culex tarsalis* and *Cx. pipiens*) weekly near (3 traps at 10 m) and far (3 traps at 150 m) from each coop location throughout the peak WNV season (July-September 2019). Chickens were tested biweekly for WNV seroconversions and serum IVM concentrations throughout the study via blood draw from the brachial vein. At the termination of the study, we fed wild-caught *Cx. tarsalis* on one chicken per flock overnight to assess the mosquitocidal activity of the blood in our study chickens for wild mosquitoes. All procedures were approved by the UC Davis animal care and use committee (protocol #20980).

Results and Discussion

Fewer chickens seroconverted in treated flocks (3/17) than in untreated flocks (11/24) and these seroconversions occurred later in the season compared to untreated flocks, indicating that there was lower WNV transmission at treated locations. However, this difference was not significant ($p = 0.069$), due to small sample sizes. A lower vector index (the product of abundance and infection prevalence) indicated a sustained reduction in risk near traps compared to far traps at IVM-treated flocks, whereas no such pattern existed around the untreated flocks, but the difference was not statistically significant ($p = 0.252$). The mortality assay using wild *Cx. tarsalis* indicated that mosquitoes feeding on treated chickens had higher mortality during the 2-3 days post-bloodmeal timeframe when IVM-related effects occurred, but this difference was not significant ($p = 0.079$). We are awaiting results on serum concentrations to further elucidate these trends and determine if we were able to reach the targeted mosquitocidal dose.

Conclusions

Oral administration of IVM to backyard chickens appears to reduce local WNV transmission, but a larger sample size is required to statistically confirm a difference in chicken and entomological metrics. Sustained oral ingestion of IVM at higher doses than previously used in poultry did not result in any adverse events highlighting the safety of this method. Taken together, oral administration of IVM provides a potential avenue for specifically targeted control of WNV in local areas. Future work aims to transition to wild birds and develop a commercial treated birdfeed for homeowner use to reduce WNV risk in their neighborhood.

Acknowledgements

We would like to thank all the Davis homeowners who hosted chicken flocks and/or mosquito traps in their yards for the duration of the study. We would also like to thank Michelle Ahn, Kurt Cramer, Emma Lonstrup, Vaz

Badiryan, Rachel Bowers Emard, Gurman Kaur, Olivia Winokur, Sarah Abusaa, Marisa Donnelly, Ania Kawiecki, Pascale Stiles, Brad Main, and Matteo Marcantonio for their hard work in coop construction, chicken husbandry and data collection. We also thank the DART Lab (Ying Fang, Sandy Garcia, and Claire Chapman), the Vector-Borne Disease Section of the California Department of Public Health (Tina Feiszli and Mary Joyce Pakingan), and the UC Davis California Animal Health & Food Safety Laboratory (Asli Mete) for providing testing for our mosquito and chicken samples. We acknowledge funding support from the Pacific Southwest Center of Excellence in Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516). The project was also supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through grant number UL1 TR001860 and linked award TL1 TR001861. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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Larvicidal treatment of ornamental bromeliads to control invasive *Aedes*

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INTRODUCTION

Tank bromeliads, which are grown as ornamental plants in California (Bethke 2019), can serve as breeding habitat for invasive *Aedes* mosquitoes (Frank 1983), including *Ae. aegypti* and *Ae. albopictus*, medically important species which have invaded southern California in recent years (Metzger et al. 2017). In addition to ornamental plantings of homeowners and in landscaping of publicly owned spaces, southern California is home to the highest concentration of commercial bromeliad nurseries in the United States. In Florida, the establishment of these mosquitoes resulted in negative publicity and reduced acceptance for tank bromeliads as ornamental and landscape plants (Gomez 2016). To help ensure the safe use of tank bromeliads as ornamental plants, mosquito larvicidal treatments are needed that are demonstrably plant-safe, effective, and efficient to apply. The goal of our study was to evaluate the effectiveness, longevity and phytosafety of insecticides applied to water-filled cavities of bromeliads to control invasive *Aedes* spp. larvae.

METHODS

The efficacy of three larvicides to control *Aedes* larvae in bromeliad tanks was studied using laboratory and field studies. The products tested included formulations of *Bacillus thuringiensis israelensis,* methoprene and pyriproxyfen against pesticide-susceptible *Ae. aegypti* and *Ae. albopictus* (USDA-Gainsville strain). SPLAT BAC®, a rewettable formulation that combines *Bti* and *Lysinibacillus sphaericus*, was tested in laboratory bioassays with and without food for the larval mosquitoes. Mortality across a range of SPLAT BAC concentrations (0.05 - 4 g/L) was compared to that in an untreated control.

Emergence inhibition of *Aedes* larvae exposed in *Neoregelia medusa* phytotelmata was studied following field applications of the insect growth regulator (IGR) Altosid®. Plants were placed overnight during truck-mounted applications of the IGR carried out by the Coachella Valley MVCD in Indio, CA. Untreated plants served as the control treatment. Plants were returned to the laboratory, fourth-instar larvae of both *Aedes* species were placed individually into water-filled cavities in each plant, and emergence of multiple cohorts was monitored across two months in two replicate studies.

The efficacy of two pyriproxifen formulations (Sumilarv 0.5G® and NyGuard®) to control both *Aedes* species was studied in laboratory bioassays using treated tank bromeliads along with an untreated control. Following treatment of the phytotelmata, fourth-instar mosquito larvae (15 larvae/plant of each of the two *Aedes* species) were placed individually in the wells of caged plants and monitored for emergence. To monitor residual activity of treatments, new cohorts of larvae were introduced and monitored for emergence every 1-2 weeks until there was no difference between control and treatment outcomes.

RESULTS AND DISCUSSION

Without food for larval mosquitoes, SPLAT BAC provided $>$ 90% mortality at 2 and 4 g/L as compared to untreated controls. However, larval mosquito mortality declined markedly when food was added to laboratory bioassays, after which there was not a strong relationship between application rate and larval mosquito mortality.

In studies of the efficacy of methoprene by applications in the field, emergence inhibition (mean EI $\sim$ 70%) differed significantly between the two treatments soon after application, was much more variable in treated plants than in control plants, and did not differ between the two treatments at two months after treatment. We suspect the variability of emergence of mosquitoes in the treated plants was related to the placement and surroundings of the plants with respect to the IGR application from the road, but this hypothesis requires further analysis.

In laboratory bioassays, two pyriproxifen (PPF) formulations were very effective and provided sustained long-term control (100% EI for $>$ 8 weeks) of adult mosquito emergence. Additional studies of PPF efficacy and phytotoxicity are currently underway.

Acknowledgments

The authors wish to thank Tony and Denise Godfrey and Olive Hill Greenhouses for donating the bromeliads used in this study; the Coachella Valley MVCD for collaboration and in-kind assistance; and MGK, Central Life Sciences, Valent Biosciences and ISCA Technologies for providing control agents and helpful discussion. We benefitted from discussions with Peter Atkinson, Nicole Colindres, Anna-Louise Doss, Jennifer Henke, Kim Hung, Jun Li, David Popko, Jennifer Williams and the San Diego Bromeliad Society. Andrew Garcia, Kimberly Leiva-Alvas, Emmilienne Colunga, Cindy Lee, Julia Perez, Adrienne Ung, and Gabriella San Jose provided technical support in the field and laboratory. Funding was provided by the Pacific Southwest Center of Excellence in Vector-Borne Diseases (CDC award #U01CK000516), the USDA IR-4 Specialty Crop Pest Management Program (award #19-025) and the UC Riverside Agricultural Experiment Station (USDA National Institute of Food and Agriculture, Hatch project 1007869).

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Impacts of larva-acquired *Aedes aegypti* microbiota on vector competence for Zika virus

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Introduction

The mosquito microbiome is an important environmental factor shaping the ability of a mosquito to transmit arboviruses such as Zika virus (ZIKV), dengue virus, and West Nile virus. However, the scale, variability, and effects of microbes, specifically bacteria, that mosquitoes encounter and acquire over the course of their life histories is poorly understood. The goal of this project is to determine the effects of bacteria acquired during larval development on the competence of the primary vector, *Aedes aegypti,* for ZIKV.

Methods

Ae. aegypti larvae were reared in either environmental water (microbe-rich, called 'Env') or standard laboratory tap water (microbe-scarce, called 'Lab') to adults which then were presented with a bloodmeal spiked with ZIKV. After a two-week incubation period, the presence of ZIKV genomic RNA in mosquito bodies and saliva was determined by qRT-PCR. 16S amplicon sequencing of mosquitoes sorted by their infection status as well as their aquatic larval habit was analyzed by QIIME2 protocols to determine if differences in microbial composition within the mosquitoes associated with differential ZIKV infection outcomes.

Results and Discussion

When presented with the same titer of ZIKV within bloodmeals, Lab group *Ae. aegypti* exhibited infection rates greater than 90% compared to Env group *Ae. aegypti* with infection rates of approximately 40%.. However, ZIKV-positive individuals for both groups exhibited similar ZIKV RNA levels. Comparisons of microbial communities showed higher microbial diversity in Env mosquitoes than Lab mosquitoes, consistent with the microbial community compositions in their respective aquatic larval habitats.

Conclusions

Taken together, these preliminary findings suggest that *Ae. aegypti* that develop in water with increased microbial diversity exhibit lower vector competence for ZIKV than *Ae. aegypti* that develop in microbe-scarce water. Although additional studies assessing this interaction in a wider range of microbial community compositions are ongoing, the findings thus far can be interpreted to mean that mosquitoes that develop in microbial rich water are less able to become infected by and transmit arboviruses than mosquitoes that develop in relatively clean water.

Acknowledgements

We would like to thank the UC Davis School of Veterinary Medicine for providing the facilities necessary to conduct these experiments. Funding for this project was provided by Training Grant Program of the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516).

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Determination of LC-50 of permethrin acaricide in the western blacklegged tick

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In the United States, Lyme disease, transmitted by the western black-legged tick, *Ixodes pacificus,* is becoming a growing problem for human health. The most recent surveillance data produced by the CDC shows a steady increase in human cases of Lyme disease, with 2017 having the greatest number of reported cases (CDC 2019). Lyme disease imposes immense health risks to humans, yet there is no widely used vaccination for prevention. The CDC recommends tick avoidance as the best way to reduce the infection with tick-borne pathogens. One recommended avoidance strategy is using 0.5% (5000 ppm) permethrin treated clothing when in tick habitat. However, this recommendation is used across all life stages for ticks, mosquitoes, and fleas which may all require different dosages. In the present study, we determined the LC-50 (median lethal concentration) of permethrin against *I. pacificus* using the Larval Packet Test (LPT) as recommended by Food and Agriculture Organization (FAO). We conducted a bioassay with 8 dilutions of permethrin in an olive oil:acetone (2:1) solution. The preliminary bioassay tested dilutions 0,5, 25, 100, 500, and 1000 ppm to inform our second bioassay. From the results of our first bioassay, the permethrin dilutions for bioassay 2 were: 0, 250, 500, 750, 1000, and 1250 ppm. The results from the combined bioassays determined a LC-50 of 508 ppm and a LC-90 of 971 ppm using Probit analysis. Permethrin is a synthetic pytheroid used against a broad range of vectors and pests, yet it is also highly toxic to non-target species including bees, aquatic insects and fish (Anderson et al). Overuse may harm non-target species, yet it is also important to determine the minimum dosage of permethrin to mitigate acaricide resistance in ticks. Other vectors including cattle ticks, *Rhipicephalus microplus,* and mosquito species, *Culex, Aedes, and Anopheles*, have already begun showing strong resistance to pesticides (Abbas et al. 2014, Liu 2015). Vectors acquire resistance through many mechanisms including increased metabolic detoxification and lowered sensitivity of targeted proteins (Liu 2015). Determination of baseline LC-50 data ensures control strategies are using proper dilutions of pesticide to avoid acaricide resistance and harming non target species. This is the first study to determine the LC-50 of a widely used acaricide on the western blacklegged tick, creating baseline data for future acaricide resistance studies. These results also indicate that the previously recommended dosage of 5000 ppm permethrin for avoidance may be unnecessarily high.

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Attraction versus capture: efficiency of BG-Sentinel trap under semi-field conditions and characterizing response behaviors for *Aedes aegypti*

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Introduction

The BG-Sentinel (BGS) is a widely used to trap *Ae. aegypti,* but little is known of its efficiency, or the proportion of mosquitoes encountering the trap that are captured. Although this trap is often deployed without any olfactory lure, it may be supplemented with CO_2 and/or a human skin odor mimic lure (BG-Lure) to increase capture rates. To improve monitoring and control of mosquito vector populations, it is useful to understand mosquito host-finding behaviors and the response to trap designs which exploit these cues.

Methodology

We tested the efficiency of capturing *Ae. aegypti* under semi-field conditions (Ritchie et al 2011) in Cairns, Australia for the original white version (BGS 1; Kröckel et al. 2006) without lures as well the blue version (Barrera et al. 2013) with and without various lure combinations ($n = 17$ per treatment). We also analyzed the flight behavior of female *Ae. aegypti* in response to the BGS in these configurations using Noldus EthoVision XT tracking software

Results and Discussion

None of the configurations tested here captured 100 % of the mosquitoes that encountered the trap. A navy-blue trap emitting CO_2 and a skin odor mimic produced the highest capture (a mean of 14 % of the total insects in the semi-field cage ($n = 100$)), but its mean capture efficiency was just 5 % (of mosquitoes encountering the trap, often more than the total in the cage). Mosquitoes often had multiple encounters with a trap that did not result in capture; they crossed over the trap entrance without being captured or landed on the sides of the trap.

Conclusion

Our research may be used to improve trap designby using increased suction and contact insecticide on the outside of the trap. These capture efficiency results also may inform abundance estimates for *Ae. aegypti* monitoring programs using capture numbers as proxy for total numbers.

Acknowledgments

The authors thank Michael Townsend and Chris Paton, Benjamin DeMasi-Sumner, Emerson Lacey and Meghan Moore for their support during data collection and analysis. The authors acknowledge funding support from the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516).

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Synthetic biology improves bacterial larvicides

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Introduction

Over the past few decades, commercial bacterial larvicides based on two mosquitocidal bacteria, *Bacillus thuringiensis* subsp. *isrealensis* (Bti) and *Lysinibacillus sphaericus* (Ls), have replaced the use of many synthetic chemical insecticides for controlling the larvae of a wide range of vector mosquitoes. These bacterial larvicides are used widely throughout the United States and many other countries, because in addition to their efficacy they are environmentally safe for most non-target organisms. However, these larvicides remain expensive. With support from NIH and the CDC, using the techniques of synthetic biology – formerly referred to as genetic engineering – we combined the larvicidal proteins that kill mosquito larvae into different bacterial strains to increase efficacy and ultimately reduce costs. The main knowledge gaps we had to fill were identifying the most highly effective proteins to use as well as to determine which genetic control factors were optimal to drive high synthesis levels of these proteins.

Methods

Many mosquitocidal proteins have now been identified from Bti, Ls, and a variety of related bacteria. These proteins, all of which destroy the stomach of mosquito larvae, include Bt proteins Cry11A, Cry11B, Cry4A, Cry4B, and Cyt1A (Park et al. 2005, Federici et al. 2007), and a potent binary toxin of Ls, BinAB (Wirth et al. 2005, Colletier et al. 2016). We first evaluated these through bioassays against a range of fourth instar larvae of different mosquito vector species, including *Culex quinquefasciatus*, *Anopheles quadrimaculatus*, and *Aedes aegypti*. We also evaluated various genetic components such as promoters, chaperone-like proteins, and RNA sequences that stabilized synthesis, which when combined together with the most potent proteins yielded high levels of the most effective larvicidal proteins. Finally, we used these combinations of proteins and genetic elements to construct highly improved larvicidal bacterial strains.

Results and Discussion

Our results showed that two strains produced using the above methods were each about 10-fold more effective than the wild type bacteria per unit weight used in commercial larvicides such as VectoBac, VectoLex, and VectoMax. The two new strains engineered were one that synthesized high levels of Cyt1A, Cry11B, and BinAB in a single strain (Park et al. 2003), and a second that synthesized high levels of Ls BinAB in Bti (Park et al. 2005, Federici et al. 2007). The Cyt1A protein is an important component of both strains because our studies have shown that this protein is responsible for delaying mosquito resistance to Bti and Ls (Wirth et al. 2005, Wirth et al. 2012, Wirth et al. 2015, Tetreau et al. 2020). We are now pursuing the development of these two strains for use in commercial bacterial larvicides for controlling both vector and nuisance mosquitoes.

Conclusions

We have demonstrated that synthetic biology can be used to develop bacterial strains that are much more cost-effective than those used in the current commercial larvicides based on wild type strains of *Bacillus thruingiensis* subsp. *israelensis* and *Lysinibacillus sphaericus*.

Acknowledgements

We thank Major Dhillon and William Van Dyke of the Northwest Mosquito and Vector Control District, Corona, CA 92881, and Michelle Brown and Steven Su of the West Valley Mosquito and Vector Control District, Ontario, CA 91761 for collaborating with us to evaluate our new larvidical strains. Financial support included partial funding from NIH award AI45817 and the Pacific Southwest Regional Centers of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516) to BF.

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Effects of short-term weather on the observed abundance of West Nile virus vectors

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Introduction

Arbovirus risk assessment programs, such as those for West Nile virus (WNV) in California, typically involve a mosquito abundance component to estimate the size of the potential vector population in an area (CDPH 2019). Weather conditions, including temperature, wind speed, and relative humidity, are known to affect mosquito population dynamics on weekly or longer time scales (Hribar et al. 2010, Chuang et al. 2011, Deichmeister and Telang 2011, Wang et al. 2011, Karki et al. 2016, Groen et al. 2017, Moise et al. 2018, Ripoche et al. 2019), but less is known about the direct impact of different weather conditions on mosquito host-seeking activity (Bidlingmayer 1985, Veronesi et al. 2012, Hribar 2017).

Methods

We collaborated with Placer and Sacramento-Yolo Mosquito and Vector Control Districts to collect mosquitoes from ten automated Biogents Counter traps (Pruszynski 2016) in a study area that included the rice-growing regions of Placer, Sacramento, and Yolo Counties during two months of peak mosquito activity in the summer of 2019. From each collection, we identified *Culex tarsalis* females, the predominant WNV vector in this area, and related these counts to the weather conditions experienced at each site during the same time periods. Data were aggregated for each overnight collection period, which is the typical time period used when estimating *Cx. tarsalis* mosquito abundance. We then characterized the relationship between overnight weather conditions and trap counts of *Cx. tarsalis* females through negative binomial regression models that adjusted for differences in average abundance among the 10 trapping locations.

Results and Discussion

Culex tarsalis females comprised approximately 70% of all mosquitoes collected during the study period, although this percentage varied by site. From the regression model, the estimated baseline counts of *Cx. tarsalis* per trap night differed by study site, ranging from 100 to over 1,000, with an average among sites of approximately 400 female *Cx. tarsalis* per trap night. These counts further demonstrated the spatial heterogeneity of mosquito species abundance within our study area, where traps were separated by a maximum of 43.6 km. On average, we found *Cx. tarsalis* activity to be greatest during nights with warmer average temperatures, lower average wind speeds, and lower daily maximum temperature. Additionally, the relationships between counts and overnight weather conditions were consistent among the different sites, indicating that a generalizable correction for these effects on mosquito activity could be applied to more accurately use trap counts to estimate mosquito abundance.

Conclusions

Our study helped explain important sources of variation in this routinely monitored component of arbovirus risk assessment programs. The results will aid vector control programs in interpreting WNV transmission risk based on mosquito abundance estimates through trap counts as well as improving estimates of adulticide efficiacy through adjustment for weather events during the application.

Acknowledgements

The authors acknowledge Tom Moore of Placer MVCD and Elizabeth Stovall and Kylie Letamendi of Sacramento-Yolo MVCDs for conducting routine trap maintenance on the Biogents Counter traps, Mirsha Torres of Placer MVCD for aiding in trap collections and mosquito counting, and the homeowners who permitted us to collect from traps on their properties.

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Using distribution models to inform surveillance for invasive ticks

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Introduction

There are approximately 50 species of ticks in California, at least eight of which bite humans (Furman and Loomis 1984). These ticks are responsible for tick paralysis as well as diseases resulting from the bacterial, protozoal and viral pathogens that these ticks transmit. But risks in California associated with ticks and tick-borne pathogens could change, especially if a novel tick species and pathogens are introduced and establish in the state. The introduction and establishment of novel ticks in California is a very real possibility, because of the importation of potentially infested hosts into the state from across the world (e.g. in 2017 approximately 675,000 cattle were imported (United States Department of Agriculture 2019)). In addition, California is an extremely diverse ecological region (Zavaleta and Mooney 2016) such that there is an abundance of potentially suitable environments available to support introduced ticks. Targeted surveillance is one of the most effective ways of preempting and responding to tick invasion (Simberloff 2003), but knowing where to target surveillance can be challenging. However, surveillance can be informed using species distribution models (SDMs), which predict the possible range of a species based on ecological variables. SDMs have already proven effective in preempting the establishment of ticks in novel ranges, e.g., *Ixodes scapularis* in Canada (Ogden et al. 2005, 2006). Herein, we used SDMs to predict the possible range in California of four species of *Amblyomma* that potentially could be introduced into the state: *Amblyomma americanum*, *A. maculatum*, *A. cajennense* and *A. mixtum*. All four of these species transmit pathogens of medical and veterinary importance and have exhibited invasive tendencies (e.g. rapidly expanding ranges), but currently have no known established populations in California.

Methods

For each of the four tick species, we used geolocation data collected from the literature and online databases, and environmental data relevant to the survival of each tick. The initial set of environmental variables included 19 bioclimatic variables (largely consisting of monthly, quarterly and annual averages of temperature and precipitation), elevation, slope, and both the average and standard deviation of normalized difference vegetation indices. Environmental variables were refined for each tick species until the models consisted of only non-correlated, biologically relevant variables. Two different datasets were used to derive bioclimatic variables. To train and test models, we used WorldClim 2.0 at 30 arc seconds resolution (Fick and Hijmans 2017). WorldClim data only span the 1950–2000 period, and although this dataset geographically and temporally matched species observations, it did not provide recent climatic information. Consequently for predictions, monthly average minimum and maximum temperature and cumulative precipitation were downloaded from the 4 km resolution parameter-elevation regressions on independent slopes model (PRISM)(PRISM Climate Group 2019) dataset for 2014–2018. Long term monthly averages were then derived for each variable and used to calculate 2014–2018 Bioclim variables with the r.bioclim module in GRASS GIS 7.6 (Neteler et al. 2012). Both WorldClim and PRISM are interpolated from weather station data (Fick and Hijmans 2017, PRISM Climate Group 2019).We performed species distribution modeling using these data and the MaxEnt algorithm to predict areas of suitable habitat in California. Predictions were visualized as binary (suitable vs unsuitable) maps based on the probability threshold at which all training geolocations were classified correctly as suitable by the model during model training (see Pascoe et al. 2019 for further details).

Results and Discussion

Habitat was predicted in California for all *Amblyomma* species except *A. mixtum* (Figure 1). Although those environmental variables which most impacted habitat suitability differed for each tick, minimum temperature of the coldest month was consistently important for all four

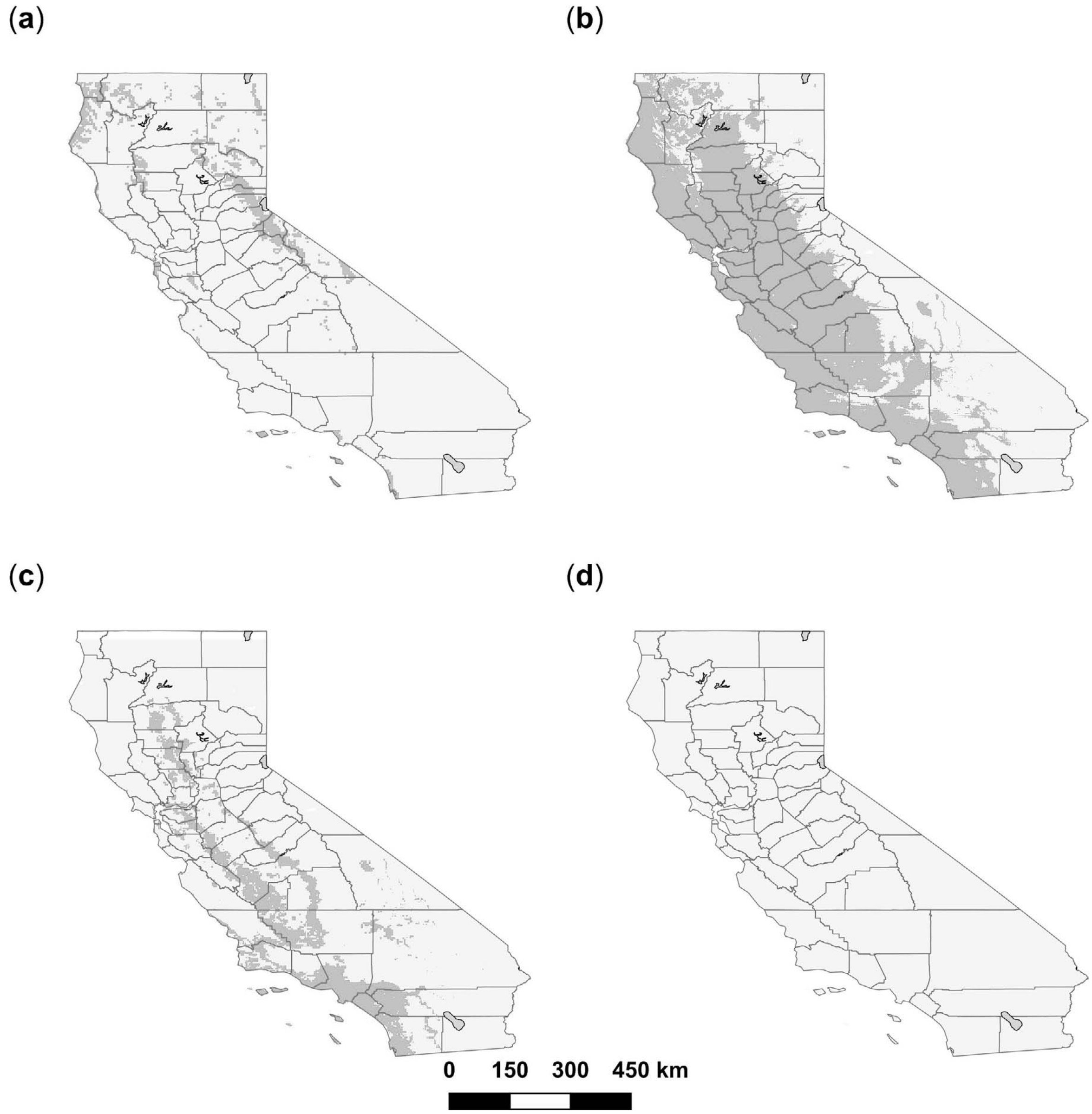

Figure 1.—Habitat in California predicted (using MaxEnt species distribution modeling) to be environmentally suitable for four exotic *Amblyomma* tick species: (a) *A. americanum*; (b) *A. maculatum*; (c) *A. cajennense*; and (d) *A. mixtum*. Areas shaded in dark gray represent potentially suitable habitat at a probability threshold at which all tick training presence geolocations were correctly classified as suitable by the model and which maximized correct classification of background geolocations. Hot arid desert was masked from suitability for *A. maculatum* and *A. cajennense*.

species. Habitat for *A. americanum* and *A. cajennense* was patchy, but animal movements throughout the state could promote subsequent invasion events in disconnected areas (Figure 1). In contrast, suitable habitat for *A. maculatum* was contiguous, potentially allowing a single invasion event to result in widespread colonization in California by this species. Concerningly, habitat was predicted to be suitable in areas that are also endemic for pathogens that the ticks can transmit. For example, the extremely contagious agent of tularemia and the agent of ehrlichiosis have both been reported in coastal and northern California, which was also considered suitable for *A. americanum* which can transmit both bacteria (Holden et al. 2003, California Department of Public Health 2017).

Conclusions

Our SDMs give insight into where four potentially invasive ticks might establish if they were to successfully invade California, for example, should they be introduced into the state with sufficient propagule pressure to proliferate. This information can help us to prioritize where to perform surveillance or mitigation activities, and provides a framework that can be applied to other tick species, including those already in California or other invasive species. It is important to acknowledge that these models are only as good as the data provided to them. Therefore, if unreliable geolocations or poor resolution environmental data are used, the models will be less accurate. Likewise, using data that is relevant to the species and interpreting it with good ecological understanding is vital, highlighting the value of data sharing and of interdisciplinary collaborations in order to take multiple approaches in mitigating the risks associated with ticks in California. Further details regarding this research can be found in Pascoe et al. (2019).

Acknowledgments

The authors would like to thank Matthew Baylis, and the three reviewers for their valuable comments on the published manuscript. E.L.P, M.M. and J.E.F acknowledge funding from the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516). E.L.P and C.C. acknowledge funding from the Biotechnology and Biological Sciences Research Council (BBSRC) US Partnering Award: Vector-borne diseases in the UK and US: common threats and shared solutions, awarded to M. Baylis (project reference BB/N021916/1). The research was partly funded by the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Emerging and Zoonotic Infections at the University of Liverpool in partnership with Public Health England (PHE) and Liverpool School of Tropical Medicine (LSTM). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, the Department of Health or Public Health England.

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Sero-survey for antibodies against Hard Tick – Transmitted Spirochetes in California Blood Donors

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Introduction

Infection with *Borrelia miyamotoi* has been well-documented in western blacklegged ticks (*Ixodes pacificus*) in California. Where the found, the prevalence of *B. miyamotoi* in adult and nymphal western blacklegged ticks is typically about 1%. In contrast, the prevalence of *B. burgdorferi* in the western blacklegged tick is about 1% in adult ticks and 3-10% in nymphal ticks (Eisen et al. 2006, Padgett et al. 2014). The prevalence and distribution of relapsing fever caused by *B. miyamotoi* in humans in California is unknown, although public health surveillance for Lyme disease in humans has identified areas in northern California, particularly coastal counties, as having a higher incidence than other parts of the state. This ample evidence of zoonotic spirochetes in California western blacklegged ticks combined with known risk areas for Lyme disease, raises the question as to whether people are exposed to *B. miyamotoi* infection to the same extent as *B. burgdorferi*. There are no commercially available tests for *B. miyamotoi* antibody, although the *B. burgdorferi* C6 antigen test has been used to screen for *B. miyamotoi* (Molloy et al. 2018), followed by more specific immunoblotting techniques, usually using the *B. miyamotoi GlpQ* antigen. One study demonstrated that 90% of sera from *Borrelia miyamotoi* patients cross-reacted with the C6 peptide antigen (Koetsveld et al. 2019). Herein, we tested sera from blood bank donors from California counties for antibodies for *B. miyamotoi* and *B. burgdorferi* to investigate the extent to which humans are exposed to *B. miyamotoi* and *B. burgdorferi*. Results of this study may help identify future areas to pursue active surveillance in an effort to obtain spirochete isolates from acute infections. The specific aims for this study were as follows:

Aim1: Measure *B. miyamotoi* and *B. burgdorferi* antibody prevalence in human sera from blood banks from selected counties.

Aim 2: Compare and contrast distribution and demographic characteristics associated with *B. miyamotoi* and *B. burgdorferi* exposure in humans in California

Methods

All human sera samples collected for Specific Aims 1, 2 were screened at UC Davis using the C6 ELISA kit developed by Immunetics Inc, Boston MA. All positive and equivocal sera samples from screening with the C6 ELISA test were subject to confirmatory testing with the Marblot (IgG) western blot specific for *Borrelia burgdorferi* and the GlpQ ELISA followed by the western blot GlpQ developed by the CDC for *Borrelia miyamotoi*. We obtained 1,700 human sera samples from Blood Systems Laboratories that included 941 samples from high risk counties and 759 samples from low risk counties. High risk counties included Marin, Mendocino, Napa, San Mateo, Santa Clara, and Sonoma whereas low risk counties included Solano, San Luis Obispo, Ventura and Orange.

Results and Discussion

Two (0.12% (Exact 95% CI: 0.01, 0.42)) of the samples were positive *B. miyamotoi,* with 1 positive sample 0.11% (Exact 95% CI 0.00, 0.59) from a high risk county and 1 positive sample 0.13% (Exact 95% CI 0.00, 0.73) from a low risk county; there was no significant difference in prevalence between high and low risk counties ($p=0.93$). The two *B. miyamotoi* positive samples came from Mendocino and Ventura counties, resulting in a county-level exposure prevalence of 2.63% (Exact 95% CI: 0.7, 13.81) and 0.23% (Exact 95% CI: 0.01, 1.30), respectively. Both sero-positive samples were females with a mean age of 24.5 yrs (SD = 10.6); one was Hispanic and the other was non-Hispanic. The prevalence detected in Mendocino County is similar to previous findings of 1.98% and 6.93% for *B. miyamotoi* in sequential years in a Mendocino community with well-documented tick exposure (Krause et al. 2018). It appears that overall in California, exposure risk to *B. miyamotoi* is quite low. However, there may be ecologic foci of greater exposure risk, similar to what is seen for Lyme disease in California (Eisen et al. 2006).

Eight (0.47% (Exact 95% CI: 0.20, 0.93)) of the samples were positive *B. burgdorferi*, with 7 positive samples (0.74% (Exact 95% CI: 0.30, 1.53)) from high risk counties and 1 positive sample (0.13% (Exact 95% CI: 0.00, 0.73)) from low risk counties; there was no significant difference in prevalence between high and low risk counties (p=0.06). Marin County had the highest prevalence of 1.27% (Exact 95% CI: 0.15, 4.53) out of the sampled high risk counties, followed by Napa 0.93% (Exact 95% CI: 0.02, 5.05), San Mateo 0.84% (Exact 95% CI: 0.17, 2.43) and Sonoma 0.52% (Exact 95% CI: 0.01, 2.84). Among the low risk counties sampled for Lyme disease, there was only one sample seropositive for *B. burgdorferi* from San Luis Obispo county, resulting in a prevalence of 0.42% (Exact 95% CI: 0.01, 2.30). Among the high risk counties, all sero-positive samples were males and among the low risk counties, the only sero-positive sample was female. The average age of the sero-positive cases in the high risk counties was 65.3yrs with a range of 56 yrs to 75 yrs of age, and the one sero-positive case from the low risk counties was 63 years old. Our study results align with what is known about the epidemiology of Lyme disease in California and the United States. The average overall incidence of *B. burgdorferi* in California over a three year period was 0.2 per 100,000 persons (Center for Disease Control and Prevention 2019, November 22). California is considered a low endemic state for Lyme disease, with infection rates ranging from 1.4 cases per 100,000 to 6.3 cases per 100,000 and an overall incidence of 0.2 per 100,000 (Center for Disease Control and Prevention 2019, November 22).

Conclusions

Overall our study demonstrated that California residents are being exposed to both *Borrelia miyamotoi* and *Borrelia burgdorferi*, but at low levels. Our results support that California overall is a low incident state for both *B. miyamotoi* and *B. burgdorferi* and even in higher Lyme disease incident counties, the risk for *B. miyamotoi* exposure is low.

Acknowledgements:

I would like to acknowledge Valerie Winkleman with Creative Research Systems for coordinating acquisition of blood bank sera samples.

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Wolbachia infections in mosquitoes of Merced County, California

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Abstract

Wolbachia bacteria are widely distributed throughout terrestrial arthropod and nematode species. These bacteria can manipulate reproduction and influence vector competence of their host. Recently, *Wolbachia* have been integrated into vector control programs for the control of *Aedes aegypti* mosquitoes. A number of subgroups and strains exist for *Wolbachia*, and they have yet to be characterized for some mosquito species in the Central Valley of California. In this study, the presence or absence of *Wolbachia,* and the subgroup and strain, were examined in mosquitoes found in Merced County. To accomplish this, mosquitoes were trapped in different habitats in Merced County and then identified to species. Ten mosquito species were abundant in the region, and these were the focus of this study. DNA was extracted from the mosquitoes. Traditional and quantitative PCR were used to investigate the presence or absence and densities of *Wolbachia*. DNA from mosquitoes positive for *Wolbachia* was sequenced to determine the *Wolbachia* subgroup and strain and by comparison to previously characterized strains. Our study detected *Wolbachia* in several mosquitoes from which it was not previously reported.

Evaluation of pyrethroid residues in California urban catch basins and associated *Culex pipiens* permethrin resistance

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Introduction

Currently, pest management in the United States relies on pyrethroid insecticides (along with organophosphates) for the control of adult mosquitoes, such as *Culex pipiens*. However, the development of pyrethroid resistance has been documented worldwide, including in the US, posing a threat to its use (Scott et al. 2015). In California, the observed frequency of the L1014F mutation, the most widespread pyrethroid-associated resistance mutation, has increased by almost fifty percent when comparing 2014-2016 observations to those before 2012 (Yoshimizu et al. 2020). Extensive research focuses on better understanding this phenomenon, including what factors in urban settings may be contributing to pyrethroid resistance in urban mosquitoes (such as *Cx. pipiens*).

The urban environment, where pesticides are often employed for structural pest control, has many features that can lead to pesticide retention and runoff. In 2016 a study in Riverside, California, found that pyrethroids and fiproles (another type of insecticide) were ubiquitous in collected dust samples, with bifenthrin and permethrin comprising the majority of pesticide concentrations (Jiang et al. 2016 a). Even if dust or other particles containing pesticides are washed away, urban layouts will generally lead them to stormwater conveyance systems, where aquatic concentrations in the parts per trillion (ng L^{-1}) range can lead to toxicity in organisms like fish and crustaceans (Jiang et al. 2016 b).

Roadside catch basins are the entry point for most urban storm drainage systems. They usually consist of a small opening to let water in, a hole connecting to the drainage system, and a sump to prevent undesirable debris from flowing further downstream. These basins, which can pool stagnant water, are known larval mosquito sources, and previous work has looked at how environmental characteristics, such as weather, vegetation, and pH, can influence larval abundance in them (Gardner et al. 2013). Less investigated is the relation urban catch basins may have with resistance development. The lack of sunlight and often-abundant organic matter, to which hydrophobic contaminants tend to adsorb, facilitates the retention of pyrethroids and other pesticides. Low concentrations of pesticides in catch basins may pose the risk of selecting for insecticide resistance, and this study aimed to examine the relationship between basin pyrethroid residues and local mosquito pyrethroid resistance levels.

Methods

Three areas-in Ontario, Woodland, and Elk Grove, California-were selected for sampling based on vector control agency availability. *Culex pipiens* mosquitoes were collected in June 2019 for use in resistance bottle bioassays. Ideally, larvae were collected from catch basin water; otherwise, gravid traps were used to catch adult mosquitoes. Mosquitoes from Woodland and Elk Grove were sent to Sacramento-Yolo Mosquito and Vector Control District (MVCD), while those from Ontario were sent to West Valley MVCD.

Environmental samples were collected once per month between June and August 2019 to observe monthly variation in pyrethroid concentrations. Initially, three basins were chosen per area for sampling, but due to some basins drying between months, additional basins were selected in July in both Ontario and Elk Grove. Selection criteria included presence of debris, water, and *Cx. pipiens* mosquitoes. Approximately 500 mL of water was taken from each basin and placed into amber glass bottles. Afterwards, approximately 100 g of solids were taken and placed into small amber glass jars. Both water and solids were collected using nonadsorbing materials (enamel, steel, or glass) and sent to the University of California, Riverside for storage at 4° C before chemical extraction.

Resistance testing was carried out in accordance with guidelines for the Centers for Disease Control and Prevention (CDC) bottle bioassay (CDC 2019). After rearing to a uniform age, mosquito populations, including the susceptible *Cx. pipiens quinquefasciatus* Say colony (CQ1) as a positive control, were tested with permethrin;

Table 1.—Pyrethroid analytes, their retention times, and the ions used for their detection and quantification.

Substance	Retention Time (min)	Ions (m/z)
D_5-Bifenthrin (surrogate)	16.4	181, 166, 165
Bifenthrin	16.3	181, 166, 165
Fenpropathrin	16.6	181, 125, 152
Lambda-cyhalothrin	18.1	181, 197, 152, 208
Permethrin	19.8	183,153
Cyfluthrin	20.8	163, 127
Cypermethrin	21.4	163, 127, 181
Esfenvalerate	23.2	167, 125, 152, 127
Deltamethrin	24.2	181, 253, 152

each bottle for Woodland and Elk Grove mosquitoes contained 43 µg of permethrin compared to 30 µg per bottle for Ontario. Mosquito survival over time was used to extrapolate an LT_{50}, which was then compared to that of the susceptible colony to obtain a resistance ratio (RR).

For extraction of pyrethroid residues, water samples were first filtered through glass fiber filters (pore size 0.7 µm) to separate suspended solids from the water (MilliporeSigma, St. Louis, MO). The water was subjected to liquid-liquid extraction with dichloromethane three times, then extracts were evaporated, transferred to hexane, and concentrated to a final volume of 1 mL. For the extraction of solids, 5 g of each sample (wet weight, separate subsamples used for moisture determination) was extracted with acetone and dichloromethane in a Dionex ASE 350 accelerated solvent extractor (Thermo Fisher Scientific, Waltham, MA). These extracts were cleaned with 1 g of Florisil (Spectrum Chemical, New Brunswick, NJ), transferred to hexane, and concentrated to a final volume of 1 mL. All extracts were injected into an Agilent 6890N gas chromatograph connected to an Agilent 5973 mass selective detector (Agilent Technologies, Santa Clara, CA) for quantification of pyrethroids. Information on the pyrethroid analytes is contained in Table 1.

Results

Resistance data is shown in Table 2. All urban *Cx. pipiens* colonies exhibited resistance to permethrin to different degrees. Overall, mosquitoes collected in Woodland and Elk Grove were more resistant than the Ontario colony, but some of this difference may be due to the difference in permethrin concentration used in the bottles. Woodland colony 3 is a notable outlier, showing a much higher LT_{50} than the other colonies. For determining resistance ratios, it is contentious whether the LT_{50} is appropriate versus LC_{50} or other concentration-based toxicity measures.

Figure 1.—Catch basin showing contents, Ontario, CA.

Total pyrethroid concentrations from June 2019 are shown in Figure 2. The most prevalent analytes were bifenthrin, fenpropathrin, and permethrin. Of the other analytes, several, such as deltamethrin, were only detected in a few of the samples. No samples were completely free of pyrethroids, with the lowest concentrations in water, suspended solids, and solids being 43.17 ng/L, 193.41 ng/g, and 44.76 ng/g, respectively. This implies that any mosquitoes interacting with the basins, either as adults laying eggs on water or as larvae developing and consuming particles suspended in water, have a substantial risk of being exposed to pyrethroids. It is possible that mosquitoes could be caught with the goal of seeing if they are bioaccumulating pyrethroids, but this requires a certain biomass of mosquitoes and additional labor, which may limit the number of vector control agencies that can

Table 2.—Resistance data in median time for mortality in min based on bottle bioassay results. CI are 95% confidence limits. Resistance ratios were determined by comparison with the local control colony. Woodland and Elk Grove colonies were compared to Sac-Yolo Control. West Valley samples were collected from Ontario, CA.

Colony	Permethrin LT_{50} (min)	95% CI	Resistance Ratio
West Valley Control	29.84	22.58 – 39.34	
West Valley 1	88.36	54.45 – 143.37	2.96
Sac-Yolo Control	18.17	14.29 – 23.10	
Woodland 1	250.47	191.41 – 327.73	13.78
Woodland 2	334.72	258.05 – 434.16	18.42
Woodland 3	1569.42	770.89 – 3195.11	86.36
Elk Grove 1	294.91	214.77 – 404.95	16.23
Elk Grove 2	284.39	212.56 – 380.48	15.65
Elk Grove 3	268.88	195.80 – 369.21	14.79

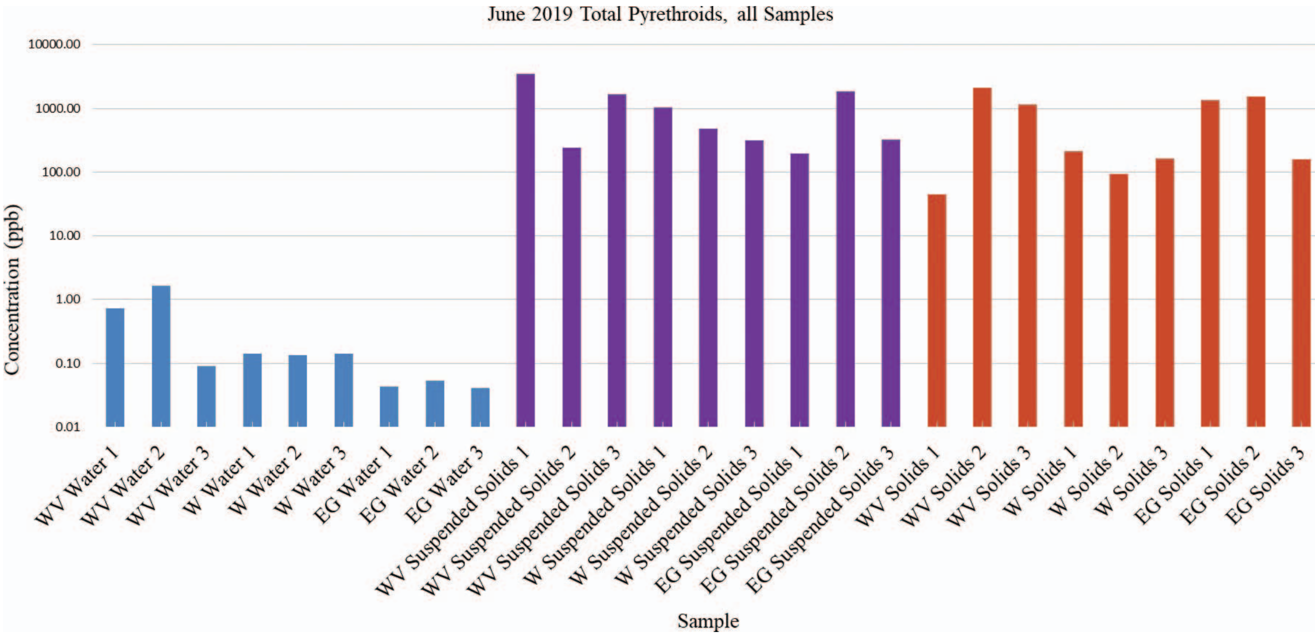

Figure 2.—Pyrethroid concentrations in parts per billion (ppb) in June catch basin samples. Water samples are represented by blue bars, suspended solids by purple, and solids by orange. WV = West Valley (samples from Ontario, CA); W = Woodland; EG = Elk Grove.

collaborate on future iterations of this project. Data analysis is ongoing for samples from July and August 2019.

Resistance ratios were compared with corresponding pyrethroid concentrations across different media and analytes (including total pyrethroids). However, associated *p*-values ($0.834 < p < 0.339$) were far too high to indicate strong evidence for a correlation; the lowest observed *p*-value was found when comparing resistance ratios with total pyrethroid concentrations in suspended solids. This indicated that either the sample size for basins (nine per month) was too low to determine a precise relationship or that there was no relationship at all between pyrethroid concentrations and permethrin resistance in *Cx. pipiens*.

Conclusions

From the current data, there was no clear association between pyrethroids in catch basins and *Cx. pipiens* pyrethroid resistance. The most likely explanation is that many more basins need to be sampled to get a picture of this possible link across California. This will require high involvement from many parties. Results do show that pyrethroids were ubiquitous in the sampled urban catch basins and that *Cx. pipiens* mosquitoes caught in and around these basins exhibited resistance to permethrin. For the continuation of this project, more consistent and robust sampling and testing will be required to obtain more conclusive results, while limiting the burden on potential collaborators.

Acknowledgements

The authors thank Zachary Cryder and Tingyu Li for technical support. Funding was provided by the Pacific Southwest Center of Excellence in Vector-Borne Diseases, CDPH Award A18-0612-S001.

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Application of Network Analysis to Identify the Risk of West Nile Virus in California

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Abstract

Machine learning can provide insights into many environmental challenges. How data is analyzed is key to properly interpreting the underlying information. Application of a data visualization technique such as network analysis with combination of machine learning for data classification can be very useful in evaluating complex problems related to vector-borne disease. Network analysis is a technique that can visualize the relationships between all pairs of data points based on multiple variables. In addition, machine learning can cluster those data that have similar characteristics. Here, we show an application of network analysis to generate the map of disease risk of West Nile Virus in California. We coupled epidemiological factors as well as surface hydrological data to understand the transmission dynamics of West Nile Virus. The results indicated that the central valley region has higher risk of infection while southern California has very low risk. This is primarily due to the landcover differences as well as the numbers of water bodies have an influence on the spatial pattern of the disease. Furthermore, the temporal analysis suggested that warm and dry years increase the infectiousness. Network analysis is useful to structurally understand the patterns of connection as well as to classify data based on the similar trend of data. Therefore, the intensity of disease risks can be identified without knowing the number of disease cases. Understanding the risks of each region would be helpful for disease control and countermeasure development.

Novel approach to preventing mosquito emergence in storm water best management practice devices

Automated larvicidal oil dispensing in BMPs

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Abstract

Recent multiyear drought conditions in Southern California have led to intensified water conservation efforts and stormwater runoff prevention, resulting in increasing numbers of stormwater best management practice devices (BMPs). These BMPs are designed to temporarily hold water for pollutant removal or ground water infiltration purposes and are often significant mosquito sources, thereby presenting an increasing problem to mosquito control agencies. Due to non-storm related runoff, some of these BMPs experience a constant high throughput of water reducing the effectiveness of residual larvicides. Novel approaches to treating these systems are needed to prevent mosquito emergence and minimize personnel time for treatments. Therefore, a timed house plant irrigation system was modified to be hung over standing water in a BMP and dispense larvicide oil. The oil's low volatility and viscosity made it ideal for application using this irrigation system. Material was dispensed at several day intervals, timed to ensure continuous emergence control. Although this study achieved proof of concept, more information is needed to determine if district-wide implementation is warranted.

Introduction

Stormwater Best Management Practice devices (BMPs) are proliferating in Southern California to help meet requirements from federal, state, and local clean water regulations (Metzger et al. 2002). The Clean Water Act, The Los Angeles County Department of Public Works Low Impact Development Plan, and the new Los Angeles County Measure W are among some of the regulations impacting the Greater Los Angeles County Vector Control District (GLACVCD, District) (U.S.C.,1972, LADWP, 2014, Safe Clean Water Program, 2018). BMPs are designed to clean stormwater before allowing the water to pass on to our waterways or to capture stormwater to allow it to infiltrate into the groundwater. Although BMPs are needed to clean and infiltrate stormwater in Southern California, many require a constant presence of water to work correctly, and it has been shown that many of these structures produce mosquitoes (Metzger et al. 2002, Kluh et al. 2001).

BMPs are designed to collect stormwater from streets, driveways, roofs, or any other impermeable surface. Stormwater runs off these surfaces into gutters, underground storm drain systems (USDS), flood channels, and eventually into the ocean. BMPs, however, catch more than just stormwater. Water from over-irrigated lawns, the washing of cars, and hosing off of driveways also is caught by the BMPs. This means that some BMPs almost constantly have a high throughput of water reducing the effectiveness of residual larvicides and resulting in the need for frequent retreatment.

To date GLACVCD has located approximately 1,500 underground BMPs in our service area. There are likely many thousands more that have not been documented and even more if you take into account all types of stormwater capture devices. Of the 1,500 BMPs that the District currently inspects, 889 are known to produce mosquitoes at some point during the year, taking a two-person crew 1.5 months to complete one inspection and treatment cycle.

It is GLACVCD's general policy to plasce the responsibility of preventing mosquito emergence from any mosquito source on the property owner; however, due to the large number of sources and the time it can take to get a property owner to do the required maintenance, the District must, in the meantime, treat the sources to reduce the abundance of mosquitoes. The increasing number of BMPs and the strain on staff hours has led to a need for an innovative solution that can help to reduce the cost of visiting each BMP frequently and utilize a single brood larvicide for a quicker kill. This need generated the idea to use an automated plant irrigation system, the Easy Grow: Easy Irrigation System manufactured by Ningbo Dewou Industrial and Trading Co. LTD (drip system), to prevent mosquito emergence by dispensing a larvi- and pupiciding surface oil at 5-day intervals.

Methods

The Easy Grow System is a battery powered home irrigation system designed for potted plants. It has a built-in timer that allows you to set a watering interval time and a

Figure 1.—The Easy Grow: Easy Irrigation System p[laced inside an old EVS trap casing with a spray bottle screwed to the bottom. A wire supports the bottle.

watering time. The watering interval time can be set to 1-12 hours or 1-15 days. The watering time can be set to 1-99 seconds. Holding down the On/Off button allows you manually dispense. It uses a diaphragm pump to dispense water from a bottle screwed to the bottom of the drip system. Vinyl tubing is provided with fittings so that one can set a system of tubing to various potted plants.

The drip system was calibrated first with water by measuring the amount dispensed in manual dispense mode over a period of 10 seconds. This was repeated three times to calculate the output rate in mL/s. Voltage was recorded from wires leading from the batteries using a multi-meter.

The drip system then was programmed to dispense at the smallest watering interval of one hour, and a watering time of four seconds to see if the output rate remained the same with a set program. The amount dispensed was measured after each watering interval and used to calculate the output rate. To test the longevity of the batteries a program was set to dispense every hour with the maximum watering time of 99 seconds. Voltage recordings were made after the drip system dispensed. On the second day voltage was recorded before dispensing and again minutes after.

The drip system was then secured inside an old EVS trap casing and hung in the BMP structure using the wire already attached to the casing. A stiff wire secured to the casing supported the spray bottle when attached (Fig. 1). The drip system was calibrated by the same method with CocoBear™ Larvicidal Oil and placed in two different BMPs chosen for their history of mosquito production and ease of access. A program was set to dispense CocoBear™ at the maximum application rate based on the water surface area and calibrated output rate. The dispensing interval was every fifth day for larvae and pupae control. The drip system was hung from a piece of rebar wedged near the access lid and the BMPs were inspected for mosquitoes and oil distribution after each programmed dispensing interval.

The first BMP located on Nada St. in the City of Downey, California, is known as the Nada St. Drain. It is a two-chamber system consisting of a sediment-catch designed to receive water from the gutter and over flow through a pipe into a 3 ft diameter dry well. The dry well, however, has a history of always holding water. Because the system is meant to clean and then infiltrate water from the street gutter, the Nada St. Drain receives a large amount of water (Fig. 2). The drip system had to be pulled from the BMP, because the water in the dry well had risen and reached the bottom of the device. After that, we were unable to prime the drip system again and a replacement system of the same model was bought, modified and calibrated but clear plastic tape was added to create a water-resistant seal.

The second BMP located on Heritage Springs Dr. in the City of Santa Fe Springs, California is a Continuous Deflective Separator device (CDS). CDS units are designed to collect trash and large debris by creating a vortex that funnels the debris into the center. For this to work the vortex chamber has to hold water and, like all CDS units, this system has trash in all parts of the system and is holding water in the lower parts. Water was observed flowing into the inlet of CDS device. Stagnant, open-surface water was observed in a part of this system, but trash clustered in the rest of the water throughout the CDS. The drip system was hung under a manhole access lid suspended in the same manner as the Nada St Drain over the open portion of water. It was eventually pulled from this BMP after repeated malfunctions.

Results and Discussion

Calibration

During calibration, lower than expected and inconsistent output were observed and a check valve was added to the draw tube to stop the flow back of water into the reservoir. Subsequently the output was still inconsistent, but this time output was higher than expected. A second check valve was added, this time on the outlet tube to stop the siphoning affect. Adding the check valves to both the draw line and outlet tube stopped the siphoning and backflow issues. In hindsight, the siphoning issue may have been preventable by shortening the outlet tube so that end of the tube would stay above the bottle, thus limiting the draw force created by gravity pulling material out of the outlet tube (Fig. 3). The check valve on the draw tube did stop a backflow of

Figure 2.—The drain and two access lids to the double chambered BMP on Nada St. in the City of Downey (right). The drip system hanging in the inundated dry well (left).

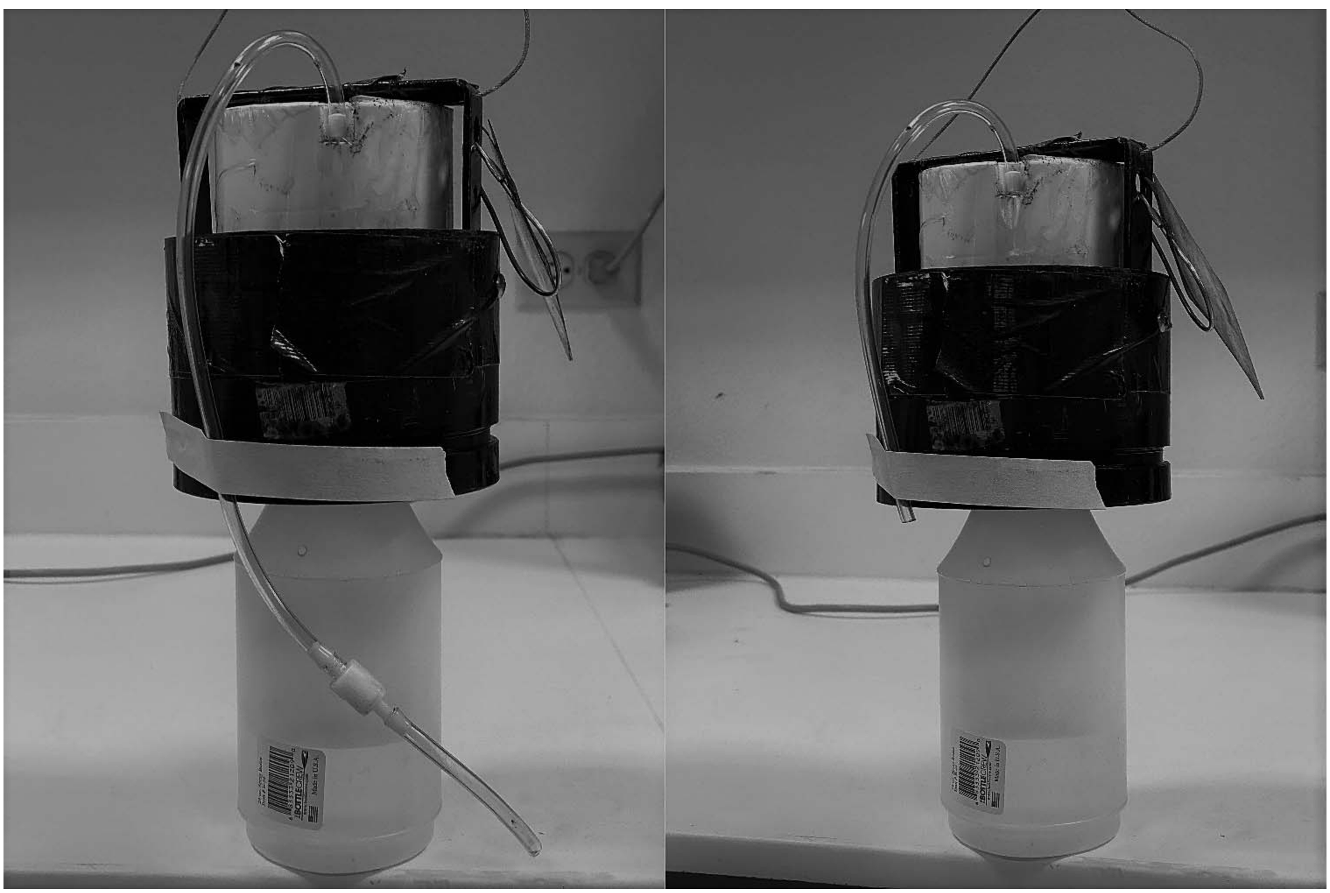

Figure 3.—The left picture shows the outlet tube and check valve with the end of the tube bellow the bottle. The right picture shows a better solution to the siphoning issue; the end of the outlet tube is above the bottle with no need for a check valve.

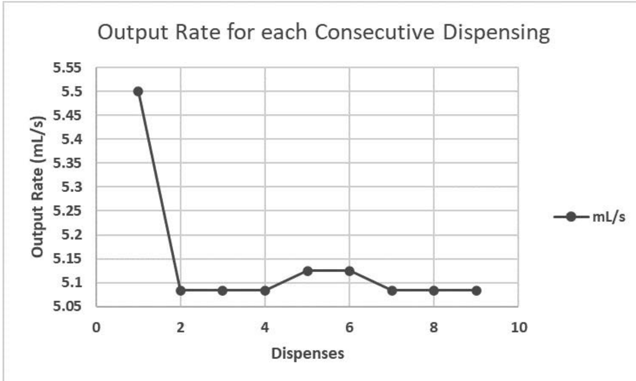

Figure 4.—Change in output rate after consecutive dispensing when a program has been set. The first dispense, after setting the program, has a higher output then the consecutive dispensing.

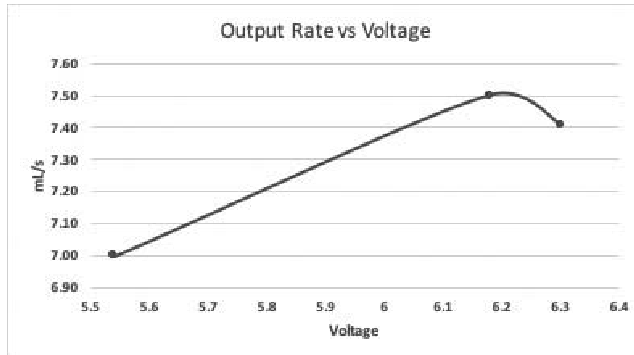

Figure 5.—In manual dispense mode the output rate drops as a function of voltage decrease. Voltage dropped by 0.76 V resulting in a 0.5 mL/s decrease in output.

material that resulted in air bubbles getting sucked in from the outlet tube; this, without the check valve, affected the volume of material dispensed and would sometimes cause a loss of 'prime' in the system. After adding the check valves the drip system was able to maintain a consistent output rate, making calibration reliable.

To set a program for automatic operation, the desired watering interval and watering time are entered; five seconds later, material is first dispensed. The first dispense of a set program has a higher output rate than the following volumes (Fig. 4). This may be a result of higher voltage at the beginning of a program. Voltage was observed to drop immediately after dispensing, but then rebound before the next scheduled dispensing. The voltage was measured from wire leads off the batteries. This fall and rise in voltage may be caused by the electronics in the drip system.

A gradual drop in volume relative to voltage was observed. During calibration using the manual dispense mode, the starting voltage and output rate were 6.3 V and 7.41 mL/s, whereas the ending voltage and output rate were 5.54 V and 7.0 mL/s., respectively (Fig. 5). Therefore a voltage drop of 0.76 V resulted in a 0.5 mL/s drop in output. In the battery longevity test, overall voltage dropped by 0.62 V over 1386 s of total watering time (Fig. 6). At an output rate of 5.0 mL/s, for example, the drip system watering time needed to be set to 8.0 s for a 100 ft^2 of water surface area. At the maximum application rate for CocoBear^{TM} of 0.43 mL/ft^2, the associated voltage drop resulted in a decreased output rate of 0.5 mL/s which would reduce the application rate by 0.040 mL/ft^2, but well above the minimum application rate of 0.26 mL/ft^2 when targeting an application rate at the high end of label specifications. Therefore, considering the total watering time and voltage drop from the longevity test, 1386 s would treat a 100 ft^2 area about 173 times without lowering the voltage enough to markedly decrease the application rate. This calculation does not take into consideration a possible voltage drop over time due to the system being switched on, having to retain its programming and dispensing every fifth day.

Field observations

When the system was deployed in the dry well section of the Nada St Drain, oil was observed on the surface of the water after each scheduled release. On the last day of the trail, mosquito larvae were observed in the untreated sediment catch chamber of this BMP. Concurrently, larvae were found in the dry well despite the fact that the sediment-catch over flows into the dry well. The dry well had oil on the surface from the last dispense. On this last observation day, the dry well had filled with water up to the bottom of the CocoBear^{TM} bottle. This was particularly interesting because there had not been a rain event to have filled it. When the drip system was pulled out, it also appeared to have gotten wet from condensation water dripping off the top of the lid. The system was unable to be primed for use again in another system.

The second drip system that was placed in the Heritage Springs BMP also dispensed a higher amount on its first dispense, but the subsequent output was found to be consistent similar to the first system. There were no larvae observed in the BMP when the drip system was installed. Five days later immediately after an application, oil was observed to have been dispensed. However, the oil had only dispersed up to the edges of the trash in the BMP. Larvae were observed in and around the trash, but not where the oil had covered the open water. Adults also were observed

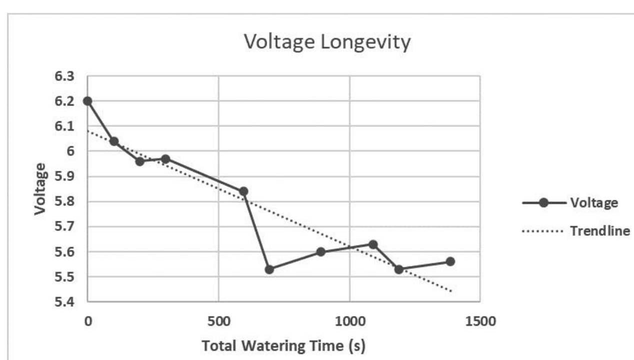

Figure 6.—Test of voltage over time. Voltage dropped immediately after dispensing and then rebounded.

flying out of the BMP when opened. The drip system remained in the BMP for another 6 weeks. No larvae were observed in any part of the BMP for the remainder of the test. This was likely due to cold and rainy weather. Towards the end of the test period the drip system began to malfunction. Upon inspection it seemed that the CocoBear™ had begun to degrade various plastic parts of the drip system. CocoBear™ had leaked inside the housing of the drip system and appears to have caused the white plastic housing to become brittle and break. It also got on the black plastic battery holder and it became ‘gummy’ and act more like a very viscus liquid as if it had melted. In addition, when CocoBear™ came into contact with moisture it became viscus. This viscus CocoBear™ and water mixture was found to have clogged the check valves and consequently was found in the diaphragm of the pump.

Future plans

For future experiments, a peristaltic pump may work better. A peristaltic pump works by progressively contracting a tube to push and draw fluid. This may work better with a more viscus fluid like a moisture ridden CocoBear™. There also are peristaltic dosing pumps used for treating aquaculture systems. This system may be a good avenue of exploration; however, one problem could be that it requires direct current. Perhaps modifying this to use a battery could be a viable solution. Another avenue of exploration would be to utilize a mist nozzle. In our regular CocoBear™ applications, the District uses a squirt bottle which can be turned down to a mist to cover a much wider area. The CocoBear™ mist droplets then cover a much wider area and disperse among trash. Incorporating a mist nozzle into this system may help overcome the challenge created by the trash in the BMPs.

The same irrigation system will probably not be continued to be studied due to the harsh nature of CocoBear™. Because the system is designed to have a bottle screwed directly to it, it will always come into contact with CocoBear™. Future systems will require testing with CocoBear™ to be sure it does not react with any of the plastic parts or vinyl tubing. The same vinyl tubing was used for draw and output lines as well as for the internal working of the system. If vinyl tubing is used with CocoBear™, clamps will be needed on the barb fittings to stop them from leaking.

Conclusion

The drip system did dispense CocoBear™ on schedule when there were not any mechanical issues. We feel that this is a proof of concept. CocoBear™ did kill larvae and pupae by blocking their access to air at the surface of the water. Having a device in a BMP that can automatically releases larvicide can help prevent mosquito emergence and greatly reduce the time and effort that mosquito control districts put into treating the vast number of BMPs within their districts.

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Utilizing artificial intelligence to improve the efficiency of our aerial swimming pool survey process

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Introduction

Alameda County Mosquito Abatement District (ACMAD) changed the process for our aerial swimming pool survey from one that had been in place for several years and was now outdated to one that was more technologically advanced and efficient. The new process decreased the number of pools that our operations staff visited on a yearly basis, reduced the time spent on each pool, and reduced the risks associated with entering private property.

Methods

This year we used the company Leading Edge for the first time to conduct our aerial swimming pool surveillance. With collaboration from Sacramento-Yolo Mosquito and Vector Control District and San Gabriel Valley Mosquito and Vector Control District, we implemented a combination of their processes that involved sending notices to residents and giving the property owners the option to text message, email, or send us a picture of their clean pool. With the assistance of Leading Edge we built upon these processes by using Artificial Intelligence (AI) to determine the quality of the pool in images from the fly over, georectifying the images based on a coordinate system so that we could apply the aerial images to the correct parcels of land on our map, and directing the importation of the images into our MapVision database.

Results and Discussion

The integration of text messaging into our database was successful with some minor issues regarding images received from residents. We received more text messages and emails combined than phone calls to the office. Our AI was lacking in accuracy because of image quality of the swimming pools which caused problems in determining water quality.

Conclusion

The District plans to rectify problems during 2019 for the next season. These new processes presented many challenges for our District but ultimately improved efficiency through technology and automation.

Acknowledgements

ACMAD would like to thank Sacramento-Yolo Mosquito and Vector Control District and San Gabriel Valley Mosquito and Vector Control District for the contribution of their processes. We would also like to thank Leading Edge for supporting the integration of the processes into their database application.

Methodologies and Lessons Learned From UAS Liquid and Granule Larvicide Calibrations

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Introduction

The Sacramento-Yolo Mosquito and Vector Control District (District) recently became licensed by the California Department of Pesticide Regulations (CDPR) to apply Public Health pesticides using Unmanned Aerial Systems (UAS) units. When calibrating for both liquid and granular larvicides, the UAS application units have many more variables that require additional considerations over traditional application equipment. With relatively few UAS units operating within the United States for mosquito control, there is little information regarding calibration techniques for mosquito control agencies to follow. This presentation discusses many of the variables and unique challenges and presents opportunities to utilize these variables when calibrating for field usage.

Methods

Due to interferences caused by the rotor-wash air movement from the UAS propellers, alternative methods of determining swath width had to be developed for both liquid and granular calibrations. For liquid calibrations, the District tested a larval bioassay transect to determine if this method would more accurately determine the effective swath width versus traditional spray card transect methodologies. Small larval cups were placed in a thirty two foot transect paced two feet apart. Water sensitive cards were placed next to each cup, to help draw a comparison between larval kill rate and droplet densities across a linear transect to help determine effective swath width. Fifteen susceptible *Culex quinquefasciatus* (CQ1 Colony from Fresno) were added to each cup immediately following the application using Vectobac 12AS. The UAS was flown in a forward motion at ten miles per hour at an above ground height (AGL) of 15 feet.

For granular calibrations the District utilized a rope with alternating colors of one foot sections of pool noodles stretched across a body of water to provide a visual image for measuring granules as they hit the water. Side cameras were aimed at the noodles and recorded video of the treatment in slow motion as the UAS flew over the transect with the granule hopper on, and recorded the overall effective swath width for each product. The UAS was flown perpendicularly over the pool noodle line at height that allowed the granular product to go out as far as possible before falling to capture the true effective swath width when go over tall obstacles.

Results and Discussion

These methods proved very useful in determining the true effective swath width using the UAS and mounted application equipment. Other variables unique to UAS equipment such as application and height above ground level and forward speed, became less problematic when the effective swath width was known.

The remainder of the discussion focused on improved workflows utilizing high grade mapping of UAS units to help determine treatment polygon, field obstacles, and determining appropriate products for the habitat.

Conclusion

UAS application equipment has proven to be a useful tool available to Districts once accurate calibrations have been documented. Although there is a learning curve associated with using this new technology, there are many benefits of using aerial data across District functions as well as improving application time and treatment areas when comparedto ground applications.

Acknowledgements

The Sacramento-Yolo Mosquito and Vector Control District Ecological Management Staff for their collaborative efforts in calibrating the District's UAS Unit.

The never-ending search for a better CO_2 baited trap

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Introduction

The Sacramento-Yolo Mosquito and Vector Control District (District) makes extensive use of carbon dioxide (CO_2) baited encephalitis virus surveillance (EVS) traps for abundance monitoring of mosquito populations as well as collecting specimens for virus testing. We are continually attempting to improve these traps, both in minimizing fiscal impact and improving efficacy. In 2019 a subset of "D-cell" battery powered traps were modified to run on rechargeable USB power packs. In tandem with this modification, the District evaluated Biogents' new BG-Pro modular trap. The BG-Pro trap can be configured as an EVS-style CO_2 trap, a CDC-style light trap or a sentinel-style trap that stands on the ground. Herein, the BG-Pro trap configured as an EVS-style CO_2 trap was compared to a standard EVS trap at multiple locations throughout Sacramento County.

Methods

The standard EVS traps used by the District are powered by three "D" cell batteries. Ten EVS traps were modified to run on rechargeable 16750mAh USB power banks with 4.5A max output (Ravpower; model #RP-PB19). The new power sources were tested for reliability and power efficiency over the course of a 26-week mosquito trapping season, with traps run twice per week.

The BG-Pro traps configured as an EVS-style trap were directly compared to standard EVS traps. The BG-Pro trap was supplied from the manufacturer with an insulated bag that held approximately 3.2 kg of dry ice pellets; very little of this ice remained in the bag at collection the following morning. Standard EVS traps have an insulated paint can that holds 1.3 kg of dry ice pellets (standard ice can) and allows for almost complete sublimation overnight. To control for the volume of CO_2 released, a third BG-Pro trap was run with a standard ice can at a subset of trapping locations. Traps were set concurrently at a total of nine rural and suburban locations across Sacramento County. At each of these sites, metal posts were placed at least 150 feet apart. To avoid placement bias, traps were rotated between posts on a weekly basis. Traps were hung one afternoon each week from August-October and collected the next day. Mosquitoes were identified to sex and species, enumerated, and the data recorded.

The total number of female mosquitoes was compared by trap type at locations where two comparison traps had both run without trap failure. To assess the species richness captured by each trap configuration, Menhinick's index (D= the number of species collected/square root of the total number of individuals collected) was calculated for each trap collection. Median trap counts and differences in mean Menhinick's index were compared by Kruskal-Wallis one-way analysis of variance on ranks. This non-parametric test was selected because datasets failed the Shapiro-Wilk normality test. Statistical calculations were conducted in SigmaPlot (Systat; San Jose, CA).

Results and Discussion

Over the course of the 2019 trap season, we tested the stability of 10 standard EVS traps converted to run on USB power packs. One power pack was damaged by crossing the polarity while retrofitting the traps, but no other battery failures were observed over the course of the season. The cost to run 100 "D- cell" EVS traps for 26 weeks was approximately $5,000/year. The cost to convert 100 EVS traps to USB power packs was approximately $3,900. This shift saved the District $1,100 the first year, and has the potential to continue to save $5,000/year for each year the USB power packs continue to function without failure. Optimally, the battery packs will last for 4-5 years. Because USB power packs vary greatly with respect capacity, we recommend evaluating suitability and capacity before selecting it to retrofit large numbers of traps.

Inspection of the mosquito trap counts plotted in Fig. 1 indicated that the BG-Pro traps run with the manufacturer supplied dry ice bag caught more total mosquitoes than both the standard EVS trap (Fig. 1a) and the BG-Pro set with a standard ice can (Fig. 1b). However, when the BG-Pro trap was supplied with the same volume of dry ice, trap counts in the the BG-Pro trap and the standard EVS trap counts were comparable (Fig. 1c). The increasing amount of CO_2 released by the ice bag supplied with the BG-Pro trap was likely the main factor in the consistently greater trap counts. Statistical analysis revealed no significant difference in median traps counts among the three different traps ($p>0.05$). Calculation of Menhinick's index for the species richness captured by each trap type (Fig. 2) followed by statistical analysis revealed no significant differences ($p>0.05$) among the trap types. By far the

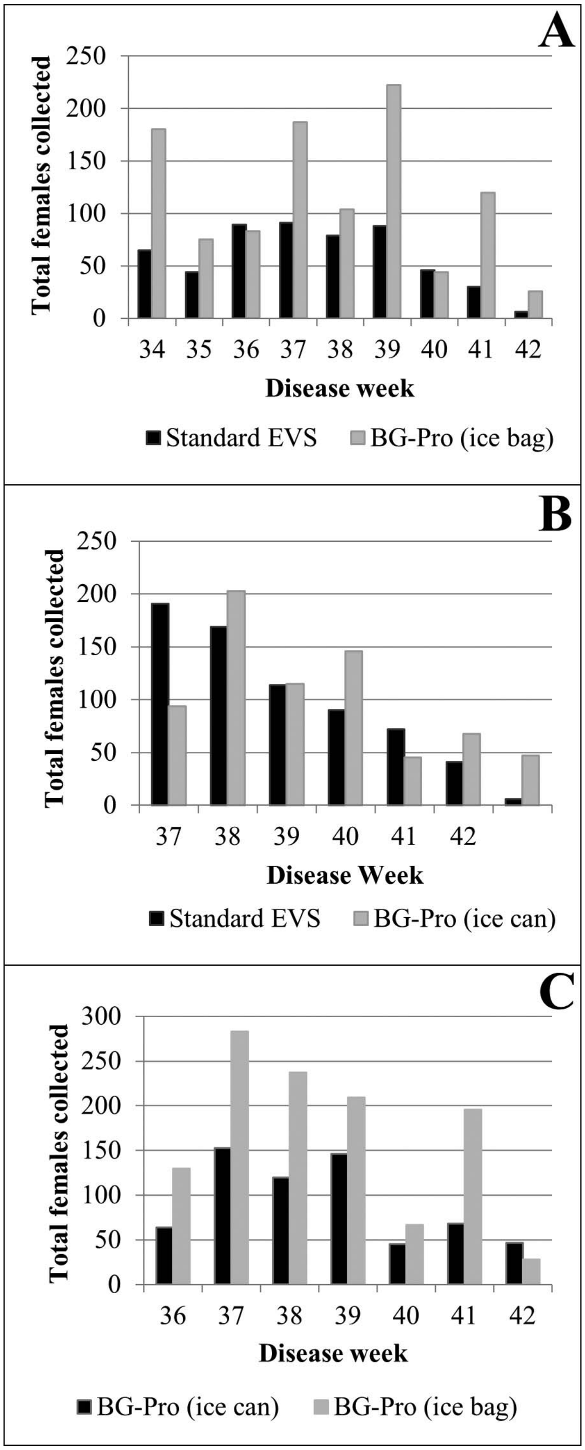

Figure 1.—The total female mosquitoes collected by week in: A. standard EVS traps versus BG-Pro traps set with the manufacture provided ice bag, **B.** a standard EVS trap versus a BG-Pro set with a standard EVS ice can; and **C.** BG-Pro traps set either with the manufacturer provided ice bag or a standard EVS ice can.

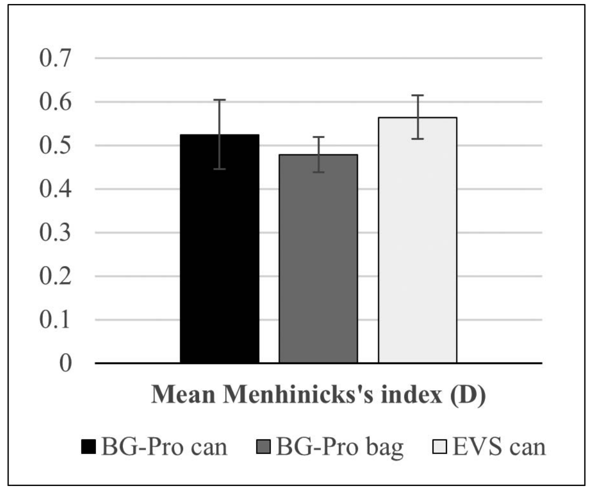

Figure 2.—Overall Species richness by trap type, mean Menhinicks index scores and standard error of the mean are shown.

mostly commonly collected mosquito species were *Culex pipiens* and *Cx. tarsalis*.

Conclusion

Conversion of EVS power sources from "D-cell" battery to USB power packs was relatively simples and efficient. The cost savings of this retrofit could be recouped within the first year of conversion. In addition, the environmental stewardship of utilizing rechargeable batteries rather than disposable batteries is important.

Overall, the BG-Pro traps operated set with the manufacturer supplied ice bag filled to capacity caught more mosquitoes than standard EVS traps. However, when the amount of dry ice was comparable to levels used in standard EVS traps, counts were similar between BG-Pro and EVS traps thereby allowing the year to year comparisons of abundance data.

The BG-Pro trap offered a unique modular trapping design, allowing one trap to be run as an EVS-style trap, CDC-style trap, or as a Sentinel-style trap. The BG-Pro trap has the advantage of interchangeable repair parts across trap types which is useful in managing surveillance programs where multiple trap configurations are utilized. Additionally, BG-Pro traps are engineered to be used with USB power packs.

Acknowledgments

We would like to thank Bryan T. Jackson (California Department of Public Health, Vector-Borne Disease Section) for technical support on this project, and Biogents for providing the BG-Pro traps evaluated herein.

High, Medium, Low: Using a Standardized County-Wide Grid Surveillance System to Prioritize Operational Areas for West Nile Virus Control in Orange County, CA

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Abstract

Following the West Nile Virus epidemic of 2014 that resulted in 278 human cases with exposure in Orange County, California, the Orange County Mosquito and Vector Control District (the District) spent the next five seasons optimizing and enhancing the District's WNV Response Plan and adult mosquito control activities. In 2019, the District developed action thresholds based on 15 years of WNV infection data and GIS analysis, and a standardized county-wide grid surveillance system (7.24 km^2) to detect increasing WNV infection rates (Vector Index) in mosquitoes and dead birds. The use of a standardized surveillance grid allows the District to prioritize operational areas for WNV control in Orange County, CA. This presentation summarized the development of the county-wide standardized surveillance grid, IVM action thresholds, and operational deployment and efficacy of the response in 2019.

Aerial Larvicide Applications with Unmanned Aircraft Systems (UAS) in Placer County

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Abstract

The Placer Mosquito and Vector Control District (PMVCD) started evaluating unmanned aircraft systems (UAS) technology for use in mosquito control in 2016. The District has two Federal Aviation Administration (FAA) part 107 certified remote pilots who operate several types of UAS in a variety of different mission types, including atmospheric measurements, visual assessment of mosquito habitat, larval mosquito detection, and aerial pesticide applications. As of August 2019, both PMVCD's UAS pilots received their Unmanned Aircraft - Vector Control Technician License from California Department of Pesticide Regulations. That license, in conjunction with the District's part 137, Agricultural Aircraft Operator Certificate, allows technicians to apply public health insecticides via UAS. Currently, the District is using a DJI AGRAS MG-1S which is configured for liquid larvicide applications. We conducted trials at different application height and speed applying water and VectoBac 12AS to kromokote cards to evaluate application rate, droplet size, deposition, and swath for the AGRAS MG-1S spray system. That data provided a good base line to successfully begin applying public health larvicides to mosquito sources throughout the county. This technology has provided another tool for smaller more precise types of aerial treatments. We will continue to evaluate UAS aerial applications by conducting more treatments, bioassays, and card trials to help determine optimal parameters for different materials and conditions.

Design, Manufacture, and Construction of an Inexpensive 3D-printed CO_2-baited EVS Trap

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Introduction

Of tantamount importance to the study of arbovirus prevalence in mosquitoes and mosquito abundance is the collection of mosquitoes in the field. Monitoring adult mosquito abundance accounts for a sizeable portion of the laboratory work for many districts. Adult mosquito abundance can be estimated using Encephalitis Vector Survey (EVS) traps, which use a light source and carbon dioxide (CO_2) to lure mosquitoes to the proximity of a small fan. If the mosquitoes are near enough to the fan, they can be pulled through the wind vortex into a closed net, and later, identified to species and enumerated. EVS traps purchased from commercial vendors cost approximately $120, when the cost of rechargeable batteries is included. The wide availability and low price of fused filament fabrication 3D printers allows for the manufacture of EVS traps that can cost less than purchasing traps. A 3D printer can also be programed to produce nearly any 3D object that can be accommodated on the printing platform, thereby enabling rapid prototyping and production of novel trap designs. The wide array of plastic filaments available for 3D printing offers a range of physical and mechanical properties such as durability, resilience to ultraviolet (UV) radiation, and biodegradability, that can be built into the trap. The 3D printed EVS trap that is described herein was printed using acrylonitrile styrene acrylate (ASA) filament which offers increased UV resistance and physical hardiness relative to polylactic acid (PLA) which is used in many hobbyist 3D printers. The trap was designed using 3D modeling software with the principal of modularity to simplify repairs and customizability to accommodate unusually shaped batteries. Using 3D printers enables the production of fully customized and novel mosquito traps without having to spend exorbitant funds for purchase.

Methods

Two types of plastic filament were used for our trap development: ASA, which offers UV resistance as well as durability, and PLA which is both inexpensive and compostable. A consideration to the selection of a primary printing material is based on the printing machine to be used. Two 3D printers were used to develop and print the EVS trap: (1) a single-nozzle, PLA-only Flashforge Finder printer (Flashforge USA, City of Industry, CA) and the Ultimaker 3 Extended with dual nozzles and a heated bed (Ultimaker, Utrecht, the Netherlands) for printing a wide range of thermoplastics. The high glass transition temperature of ASA requires the use of a 3D printer with a heated bed to prevent warping of the object being printed. Three different 3D-modeling software applications were utilized for this project: Tinkercad (Autodesk Inc., San Rafael, CA), Blender (Stichting Blender Foundation, Amsterdam, the Netherlands), and AutoCAD (Autodesk Inc., San Rafael, CA). The free-to-use software Tinkercad is user-friendly, but it cannot make intricate, mechanically precise models. Blender, while free, requires greater effort to learn and is used more frequently as artistic modeling software rather than an engineering tool. AutoCAD was utilized primarily to construct 3D models of the EVS trap parts (Fig 1A.). Thingiverse, available at thingiverse.com, is a repository of 3D models. It is a free service, but it requires one to upload the designs to the website. Once all seven trap parts had been designed and printed, the additional parts were purchased and assembled to produce the completed 3D printed trap (Fig. 1B). The required additional parts, available from a variety of vendors, include: fan motor (model RF500TB-14415; Solarbiotics, Calgary, Canada), 4 blade 74 mm diameter fan blade (BioQuip Products, Rancho Dominguez, CA), ultra bright white 12V light-emitting diode, a universal serial bus (USB) cable to deliver the necessary energy from the battery, 20000 mAh lithium-ion polymer (LiPo) double USB battery (model 26111700; AiBOCN, Wilmington, DE), a cable from which the trap could be hung, nuts, screws, and 140 mm threaded rods to provide reinforcement and mounting. The 3D printed trap was compared to a conventional Heavy Duty EVS CO_2 Mosquito Trap (BioQuip Products, Rancho Dominguez, CA) to determine the suction force it produced using a Kestrel 5500 Weather Meter (Kestrel Instruments, Boothwyn, PA). The quantity of CO_2 released from the EVS traps was assessed using a CO_2 monitor (pSense High Accuracy ($\pm$ 30ppm) Portable CO_2 Meter,Senseair AB, Delsbo, Sweden). The mean and standard error of the mean (SEM) of two measurements at each distance was calculated and graphed using Prism Software (version 8.4.2; GraphPad Software, San Diego, CA)

Figure 1.—(A) 3D model of the EVS trap produced using AutoCAD software. (B) Assembled 3D printed EVS trap.

Results

All seven trap parts were printed using the Ultimaker 3D printer in 14 h. Once the parts were printed and other materials collected, the assembly of a complete 3D trap took 15 min. The total cost of a completed 3D trap was less than $54. The first test performed on the trap was an assessment of the fan power compared to the original, purchased EVS trap. Using a wind meter, the original EVS trap had an inward wind speed of 0.85 m/s whereas the 3D-printed trap was 1.4 m/s. The wind speed leaving the trap was 2.2 m/s for the original EVS trap and 3.1 m/s for the 3D-printed EVS trap. The second test was to assess the dispersal of CO_2 from the center of the traps. CO_2 concentration was measured 60 min after dry ice was placed into a standard EVS trap bucked that was suspended

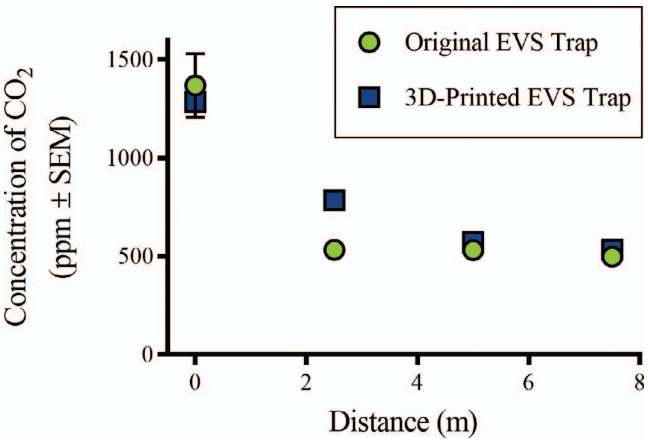

Figure 2.—The dispersal distance of CO_2 from the original EVS trap and the 3D-printed EVS trap.

above the traps at distances of 0 m, 2.5 m, 5 m, and 7.5 m. The results suggest that the original and 3D-printed traps disperse CO_2 with similar efficiency (Figure 2).

Discussion

A complete comparison of the strength of the two thermoplastics we used to 3D print EVS traps will require more study. However, during our brief use of the two traps in the field, PLA seemed to perform as well as ASA. The strength of PLA may be adequate in the short term but become weaker upon repeated, long term use. Because the cost for a printer that utilizes only PLA is much less than one that uses a broader range of thermoplastics, the PLA-only 3D printer is an appealing option to consider for those wishing to minimize cost. In the design of the 3D model, AutoCAD was preferred to the other modeling software that was evaluated. The amount of constructive control of the sculpted object as well as the intuitive interface for the formation of mechanical objects made AutoCAD the ideal application for trap design. Price was the driving factor for selecting the USB LiPo battery. The ubiquity of rechargeable USB LiPo batteries has driven down cost while increasing the power capacity. Moreover, LiPo batteries maintain their capacity for more charge-discharge cycles than the typical nickel-cadmium rechargeable batteries that are often used for the original EVS traps. In summary, we demonstrated that design, manufacture, and construction of an EVS trap can all occur on-site at a low cost using a 3D printer.

Acknowledgments

We appreciate our colleagues at the Alameda County Mosquito Abatement District, for being supportive, kind, and a hotbed of creative ideas.

CalSurv Application Programming Interface (API) and Tick module

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Over the last decade, the method for exchanging data between mosquito control agencies' in-house software and the CalSurv Gateway system has involved web services that communicate with the underlying databases via JavaScript Object Notation (JSON). The web services operate in parallel to the CalSurv Gateway website (CalSurv Gateway 2020) and have a distinct set of code that allows for import and export of surveillance records. This feature has been valuable for avoiding redundant data entry by agencies that use their own software for data entry or analysis. The current web services present challenges for ongoing development and maintenance, because they are not tightly integrated into the CalSurv Gateway's broader architecture.

Application programming interfaces (APIs) are a more modern way to connect to 'back-end' databases. One analogy is to consider APIs as being like a menu in a restaurant, and the waiter is the API server. The menu (API) defines what can be ordered, and the waiter (API server) conveys the order to the kitchen (database) who prepares your order from the available ingredients (data). The customer (end user or third-party software) does not need to interact with the kitchen directly or understand how the ingredients come together to result in the final product. All the end user cares about is that the 'food' ordered (data) are as requested and served in a timely manner. In modern APIs, requests to the database are often in the form of web addresses (URLs) that include strings of filters that represent the specific data desired.

Over the coming year, we are developing a new application programming interface (API) for the CalSurv Gateway. Unlike our existing web services that serve only external software, the new CalSurv Gateway API will connect all CalSurv Gateway-related applications to the database, including our own Gateway and Maps websites (CalSurv Gateway 2020; CalSurv Maps 2020). Having the API server as the interface for all development will streamline the architecture and make future development and maintenance straightforward for our team and third-party software developers.

The first element of the CalSurv Gateway website to utilize the new API will be a new tick surveillance module to be released in 2020 that will allow users to enter and manage data on tick collections and testing. The tick module will be similar in concept to the existing CalSurv Gateway modules for arthropod collections and testing, but will be tailored to the methods used for ticks. Many of these methods involve collection methods (e.g., dragging flannel cloth or extraction from animal hosts) and sampling units (e.g., spatial area) that differ from those for mosquitoes and other biting flies.

Acknowledgements

We thank our partners including the state of California, California Department of Public Health, and the Mosquito and Vector Control Association of California for the long-standing support that makes the CalSurv Gateway and Maps websites possible. We also acknowledge funding support from the National Aeronautics and Space Administration's Applied Sciences Program in Health and Air Quality (Grant NNX15AF36G) and the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement U01CK000516).

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Gateway gotchas: a review of data entry methods, analysis tools, and common errors for the CalSurv Gateway

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The CalSurv Gateway (CalSurv Development Team 2020a) is the web-based platform used for management and analysis of data on mosquito surveillance and control throughout California. The system has been in continuous use since its launch in 2006 and now serves 83 vector control and public health agencies in California, with > 900 registered users. The collection of individual web pages that make up the CalSurv Gateway website has grown to > 100 pages of content. This abstract highlights some commonly overlooked features and available documentation that may be helpful, even to long-term users of the CalSurv Gateway.

Forgot username or password?

The panel on the right side of any CalSurv Gateway page often displays helpful information. One example is the "Login Help" on the right side of the initial CalSurv Gateway screen (CalSurv Development Team 2020). This panel provides an easy way to retrieve your username or reset your password anytime.

Need to add a user in your agency?

The CalSurv development team often receives requests from vector control district employees who want to be added as new users within their agency. We do not have the authority to manage an agency's users. Instead, user privileges are controlled by those users with "Agency Manager" privileges within each agency. These individuals manage other users' accounts within the agency and can create new user accounts. For agency managers, new users can be added by visiting Settings → My Agency → Manage Users on the CalSurv Gateway.

Got issues?

CalSurv Gateway users will notice that a new tab was added to the website in late 2019 to indicate "issues" with existing data. This is a result of new quality control checks to improve data integrity. The most common issues are (1) collections that were entered into the CalSurv Gateway prior to the date when the trap was collected, or (2) collections or tests from spatial locations that fall outside of an agency's boundary. In some cases, these are due to data entry errors, and the CalSurv Gateway's list of issues offers a link to view or edit the record. Once corrected, there is a link to submit the issue as resolved to clear it from the list. Issues that do not require any correction may be submitted as resolved with no further action.

Can I print lab worksheets for mosquito collections?

The CalSurv Gateway has an option to produce easy printable worksheets for use in the laboratory to record collections of mosquitoes or other arthropods. This capability can be found under Arthropod → Abundance → Worksheets. These worksheets mimic the format of laboratory worksheets that have been used by California mosquito control agencies for decades, which were created originally by the California Department of Health Services (now California Department of Public Health). The CalSurv Gateway's implementation allows agencies to customize these worksheets with locally relevant species and specific lists of surveillance sites that can be saved and reprinted anytime.

Need more help?

For more information on the issues above as well as other CalSurv Gateway capabilities, please visit our new documentation page (CalSurv Development Team 2020b). We also post new training videos when available on our YouTube Channel (CalSurv Development Team 2020c). If your question is not answered in the documentation above, feel free to send an email to the development team anytime at help@calsurv.org.

Acknowledgements

We thank our partners including the state of California, California Department of Public Health, and the Mosquito and Vector Control Association of California for the long-standing support that makes the CalSurv Gateway and

Maps websites possible. We also acknowledge funding support from the National Aeronautics and Space Administration's Applied Sciences Program in Health and Air Quality (Grant NNX15AF36G) and the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement U01CK000516).

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CalSurv Maps

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The CalSurv Gateway is a web-based data management and analysis system that is California's official repository for data on the surveillance and control of vectors and the pathogens they transmit (CalSurv Development Team 2020a). The system has been online since 2006 and is made possible by a partnership of the Mosquito and Vector Control Association of California, California Department of Public Health (CDPH), and the University of California, Davis. The system gives users a variety of tools for analysis and visualization that make surveillance data more accessible for decision-making. For example, users can compare present trends in mosquito abundance or arbovirus activity to those of prior years using online calculators.

CalSurv Maps is a companion website that displays real-time surveillance data on a series of maps that inform vector control districts, public health agencies, and the general public (CalSurv Development Team 2020b). The interface displays a series of five interactive maps, each with its own graphs showing time trends, along with relevant user-defined filters. Over the past year, we have added new maps and made a number of enhancements that are summarized below.

Arboviruses. Maps of arbovirus activity show all detections of arboviruses in mosquitoes, sentinel chickens, and dead birds. Users can activate the different map elements (viruses and surveillance methods) by clicking the buttons with corresponding colors. The time slider defines the start and end dates for the data shown, allowing users to visualize data going back to 2003, which was the year West Nile virus was first detected by enzootic surveillance in California. Clicking on the map's data points shows graphs of changes over time for the corresponding city, and site visitors can use the map's polygon tool to draw a custom boundary around any area of interest to see trends for that area over time. Each data point is randomly shifted by a small distance up to 0.4 km in a random direction to obfuscate exact locations of surveillance sites while preserving the overall spatial pattern of arbovirus detections. The "show surveillance" button in the map's lower left corner overlays a heatmap showing the relative testing effort when activated.

Invasive *Aedes*. Maps of the three invasive *Aedes* species found in California (*Aedes aegypti*, *Aedes albopictus*, and *Aedes notoscriptus*) show current distributions and time trends for each species. Because these species are primarily found in and around human dwellings, the spatial units for this map are cities and census-designated places (CDPs). Some very large cities such as Los Angeles are further subdivided into communities with place names recognized by the city. Cities in green are those with a history of collections using *Aedes*-specific trap types (Biogents Sentinel traps, oviposition traps, Biogents GAT traps, or autocidal gravid traps) without detecting the chosen invasive species, and cities in red, green, or yellow indicate cities with history of detecting that species within the chosen date range. Users can 'mouse over' individual cities to see the time since the last detection of the mapped species, and clicking the map reveals a graph and additional details about the history of detections in the selected city. For *Ae. aegypti* and *Ae. albopictus*, graphs include model-based predictions for seasonal variation in reproductive suitability (population growth as a percentage per day) based on weekly temperatures from NASA's Terrestrial Observation and Prediction System (TOPS) or North American Land Data Assimilation System (NLDAS) gridded products (Nemani et al. 2009, Xia et al. 2012).

Dengue/Zika Risk. Risk for dengue and Zika virus transmission is presented as the basic reproductive number (R_0), which estimates the average number of secondary infections that would be expected if local transmission of dengue or Zika virus were to occur within a particular city or CDP. These viruses are transmitted primarily by *Ae. aegypti*, and the densities of *Ae. aegypti* that inform the R_0 estimates vary between 0 and 5 adult females per person based on fitted statistical curves for each region (southern coastal, southern deserts, and southern Central Valley) that represent the typical seasonality of adult female *Ae. aegypti* abundance from mosquito trapping data. Other parameters used to estimate risk are the predicted *Ae. aegypti* biting rate and daily survival, and the extrinsic incubation period for dengue and Zika viruses (Chan and Johansson 2012, Winokur et al. 2020), all of which are estimates as a function of temperature using the TOPS and NLDAS data described above. Higher values of R_0 indicate greater risk, and because of the assumptions involved in the model estimates and their calculations at the relatively coarse scale of cities and CDPs, these estimates should be regarded only as relative indicators of places and time periods with higher risk. The models used are not capable of representing potential heterogeneities at finer scales of neighborhoods or households.

Mosquito Abundance. In late 2019, new interactive maps and graphs were added to show average adult mosquito counts per trap-night by mosquito control agency. These visualizations were intended to increase utilization of the data from the weekly Adult Mosquito Occurrence Report that was distributed by the Vector-Borne Disease Section of CDPH for several decades through 2019 (CDPH 2020). The maps and graphs can be filtered by species, degree of urbanization (Barker et al. 2004), and trap type, and users can view all years dating back to 1952, which are the earliest mosquito abundance records stored in the CalSurv Gateway. At present, abundance maps and graphs are available only to users with CalSurv (or VectorSurv for other states) login credentials.

Insecticide Resistance. New maps and graphs of insecticide resistance and usage were added in early 2020. Colors of mapped points indicate the resistance status of field populations based on percentage knock-down in CDC bottle bioassays (Brogdon and McAllister 1998) at the user-defined diagnostic time. Clicking on bioassay data points generates a menu showing the bottle bioassays conducted on mosquitoes from the chosen site, and the user has the option of adding the individual bioassays to a plot showing mortality over time. The bioassay mortality graph then has additional interactive that allow for further investigation of the data.

The maps also display publicly available pesticide usage data that are imported into the CalSurv databases from the California Department of Pesticide Regulation's Pesticide Use Reporting database (CDPR 2020). When 'zoomed' out to the scale of California, users see pesticide use data by county, and as the user zooms in on the map, the displayed data resolves into more granular scales of townships (6×6 mi) and sections (1×1 mi). Clicking on individual areas of the pesticide use map reveals graphs of time trends in annual pesticide usage from 2000 through the most recent year available (presently 2017). Time sliders allow for restriction of the data to certain years for both bottle bioassays and pesticide use. Buttons allow for filtering bottle bioassays by mosquito species and technical grade product, and pesticide use may be filtered by pesticide class (pyrethroids, organochlorines, botanicals, organophosphates, and/or carbamates).

Sharing Tools. Clicking the "Share" button in the upper right corner of each CalSurv Maps page displays the options for sharing the page's visualizations. These options include copying a link to the current page as configured, including user options and zoom level, downloading the map or chart as image files (e.g., for inclusion in publications or agency reports), or copying iframe code that can be embedded in local agency websites. The last option could be useful for mosquito control agencies or news media who wish to provide a live view of CalSurv Gateway data in their local area through their own website.

Acknowledgements

We thank our partners including the state of California, California Department of Public Health, and the Mosquito and Vector Control Association of California for the long-standing support that makes the CalSurv Gateway and Maps websites possible. We also acknowledge funding support from the National Aeronautics and Space Administration's Applied Sciences Program in Health and Air Quality (Grant NNX15AF36G) and the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement U01CK000516).

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Funding to Fight the Bite: Implementing Revenue Mechanisms 101

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Abstract

Adequate funding for control of invasive mosquito species should be a component of any budget of local mosquito agencies across the State. This presentation will discuss how to raise additional revenues to fund current services, to better prepare to combat new invasive mosquito species, and to help prevent emerging diseases. Discussion includes a comparison of funding alternatives, with particular emphasis given to parcel taxes and benefit assessments. The importance of working closely with the community and elected officials effectively messages your service needs. Our presentation covers the findings from surveys and opinion research projects previously conducted by our firm.

Invasive *Aedes* Infestation Forces Service Model Changes for Orange County Mosquito Control District in 2019

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Abstract

After back-to-back West Nile virus epidemics in the fall of 2015, the Orange County Mosquito and Vector Control District (OCMVCD) began receiving multiple public reports of black and white day-biting mosquitoes. Within six weeks, OCMVCD confirmed the presence of invasive *Aedes* mosquitoes at ten locations in nine Orange County cities (*Aedes aegypti*, 8 locations; *Ae. albopictus*, 2 locations). The rapid and intense spread of invasive *Aedes* throughout much of Orange County taxed OCMVCD resources as residents demanded service inspections seeking relief from these aggressive mosquitoes. Service requests for mosquito bite complaints increased on average 20% from 2015 - 2018. To manage the demand and better allocate resources, OCMVCD evaluated relevant aspects of its service model and outlined a five-year operational strategy for the service request program. In 2019, OCMVCD implemented the initial phase of changes to the program which included: enhanced education resources aimed at providing resident empowerment tools, adding call center support staff, repurposing seasonal staff to decrease workload burden on Zone Inspectors, and implementing a scheduling and dispatch system for improved workload distribution. This presentation discussed the results of the first year of implementation of the service model changes and highlighted plans for 2020 and beyond.

Division of Labor in the Age of *Aedes*

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Introduction

The invasion of *Aedes* mosquitoes into Los Angeles County has changed the way vector control districts operate. Although agencies must continue to focus on *Culex* species and West Nile virus control, the bite pressure from *Aedes* mosquitoes ensures that these invasive pests get more attention from the public and media. To address the changing demands and an ever-growing scope of work, the Greater Los Angeles County Vector Control District [GRLA] divides staff into teams that specialize in operations to surveillance to community outreach. The current presentation addresses how labor and responsibilities are divided to be more efficient and effective at fulfilling the district's mission to protect the public health.

Methods

The GRLA conducted internal strategic planning sessions and budgeted for additional staff to meet growing service demands. A look at the District's organizational structure in conjunction with assessment of work scope led to a better definition of duties amongst teams and individuals. Some divisions of labor existed prior to the discovery of *Aedes* mosquitoes; however, the proliferation of *Aedes* in recent years has expedited implementation of additional positions and roles.

Results and Discussion

The presentation addresses how responsibilities are divided between and within district departments and describes the strengths and weaknesses of this organizational structure.

Conclusion

The district will maintain and refine these organizational strategies based on future data and assessments, which will also inform future budgeting decisions and goal setting.

Meeting the Demand for Service in the Age of *Aedes*

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The introduction of *Aedes* mosquitoes into California has created new challenges for vector control agencies. The San Gabriel Valley Mosquito and Vector Control District(District) has detectedthe presence of invasive *Aedes* species in every city within its jurisdiction. The aggressive nature of *Aedes aegypti* and *Aedes albopictus* and their preference for a human host has significantly increased the biting nuisance residents experience from mosquitoes. As residents find it increasingly more difficult to enjoy outdoor spaces, they turn to the District for help.

The public demand for service has grown exponentially since the establishment of *Aedes* in the District's communities. Between 2016 and 2018, the number of service requests submitted to the District increased by ca. 90% each yea, for a cumulative increase of 272%. This dramatic rise in demand has strained the limited resources of the District and has forced innovation in response.

As a result, the District has shifted focus and resources to prioritize long-term, sustainable, and pro-active strategies that empower residents to control mosquitoes in their own environment; a focus on education and outreach that promotes positive habit change and a shared responsibility.

To shift focus in a responsible way, we reviewed our existing program and decided to measure options based on the following goals.

- Return focus to our mission to prevent vector-borne disease. This means a renewed focus on West Nile virus and a prioritization of preventative maintenance.
- All *Aedes* response must focus on education, because a one-time source removal or chemical treatment does not provide a solution. We must promote meaningful habit change that empowers residents.

It is also important to us that residents have a positive perception about the work that we do and the contributions we make to public health.

Using these goals as a lens, we evaluated our service request process and found the following opportunities for improvement:

- By submitting a service request, many residents thought they were requesting a pesticide treatment and that service would make their mosquitos problems go away. These popular false expectations created disgruntled residents.
- Many requests early in the season were relatedto midge or crane fly presence.
- Many residents were not present during property inspections.
- Many return visits showed no real habit change or any motivation from resident to take ownership of the situation.
- Residents had absolutely no stake in the process
- Because the District was pulling resources from every corner to address service requests,the District completely lost the ability to direct resources for proactive disease prevention. Science was no longer directing our resources; the most vocal and not necessarily the most needy residents were utilizing the most resources.

To address these inefficiencies and reprioritize our efforts, the first thing we did was eliminate the prominent, catch-all "Service Request" button from our website. We replaced it with several individual response programs that could satisfy specific needs. In the old service request model, we gave residents entirely too much latitude to tell us when, where, and how they wanted service. It locked us into a commitment where the residents expectations dictated the terms.

To re-gain control of the process, we replaced our single, one-size fits all service request option with a new suite of support services that include:.

- District Tip Line
- Property Consultations
- Mosquito Fish
- Education and Outreach Programs
- Enhanced Neighborhood Support

District Tip Line

With our new tip line, residents can call or go online to share information that will help make decisions that direct the District's resources. Most of the traditional service requests now come through the Tip Line. The difference is that instead of requesting service, residents are now providing information. This small but important difference allows us to better communicate what we are going to do with the information they give us.

When using our website to submit a tip, the resident is directed to a webpage specifically designed to define expectations. The webpage contains:

- A video explaining the tip process and what the resident can expect after submitting a tip.

- A "Shared Responsibility" plug. Shared responsibility is a central theme in all our outreach but here specifically it reminds people that they have a role to play, a "responsibility" in this interaction.
- Prominently displayed education about crane flies and midges to prevent unnecessary demand due to misidentification.
- An explanation of our priorities and our mission. It lets the resident know that the information provided will be used in the fight against mosquito-borne disease and prepares them for the possibility that they may not be contacted or followed up with directly depending upon the type of information they are providing.
- An explanation of resident responsibility. Specifically, the idea that residents need to commit to long-term solutions for sustained mitigation and that efforts by the district on private property provide only temporary stopgaps.
- Headlines and links to detailed articles explaining why the District does not just "come out and spray."
- An interactive map that displays all of our education and outreach efforts and emphasizes our commitment to long-term habit change.

Property Consultations

The property consultation is our response to a majority of the *Aedes* related nuisance calls, although, we no longer differentiate species as a condition of response. Consultations include:

- A scheduled visit from a vector control specialist
- An introduction to mosquito biology that includes life cycle and habitat.
- A hands-on preliminary property inspection that includes habitat identification and elimination, mosquito bite prevention and exclusion, and finally a long-term preventative maintenance plan.
- An affirmation of shared responsibility and suite of neighborhood outreach opportunities.

This information is summarized in a yellow handbook that the consultant uses as a visual aid throughout the process and that is left to help guide the long-term maintenance plan. During the process the consultant is using a tablet to fill out this consultation report that informs the resident exactly what was found and what needs to be done. This report is then printed, using a portable printer, in color, on quality paper and left with the resident.

We started implementing these ideas during the 2018 season. We introduced property consultations in June of 2019. Since then we have seen a significant 31% reduction in service requests even as *Aedes* populations continue to increase. This is the result of a deliberate and concerted effort to provide better information to residents before, during, and after submitting a service request. Positive results through shared responsibility frees up resources that the District can shift toward its proactive and preventative efforts to minimize the threat of disease.

The 2019 season was the first season in years that we did not have to redirect staff from regular work to cover service request demands. Also, for the first time in years, the District received less calls from angry residents, and not one that I did not resolve by walking them through explanations on our website. We still have a lot of work to do in this systemic shift. There is plenty of room to further develop these ideas, but I feel like we are on the right track. We have reduced service request demand, increased the quality of service, and started building realistic expectations around the core issues associated with *Aedes* and what the Districts role will continue to be in the future.

Which Way Did He Go? Using fluorescent tracking powder to locate cryptic rodent burrows and structural access points

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Abstract

Smaller ground dwelling and burrowing rodents (e.g., chipmunks and mice) are readily trapped, but locating their burrows is time consuming and often impractical. In 2019, we evaluated the utility of fluorescent tracking powder for locating cryptic rodent burrows and structural access points. Our observations indicated that this method may be useful in determining the locations of cryptic rodent burrows when burrow dusting is needed during plague epizootics, and/or identifying structural access points used by mice while assessing hantavirus exposure risk.

The importance of dissolved oxygen for high yield production of quality *Gambusia affinis* and overall earthen pond health

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Introduction

The goal of this project was to increase mosquitofish production within the confines of our District's facilities to meet the annual demands of providing fish for almost 50,000 acres of rice fields and wetlands, in addition to the numerous urban sources. The District's main office is located in Elk Grove with residential and commercial property limiting expansion, thereby highlighting the importance of improving the efficiency of fish culture to increase production.

Methods

Methods used to increase pond health and the production of mosquitofish were based on monitoring water quality by checking turbidity, temperature and dissolved oxygen (DO) and making adjustments as needed. DO was monitored starting the first week of January using a Milwaukee MW600 dissolved oxygen meter. Adding supplemental aeration created more usable oxygen for the desired increase in biological load and overall pond health. Our Keeton Industries Solaer® aerator SB-1-2 was run from 1700 – 1000h when photosynthesis and therefore oxygen production tends to decrease. Mosquitofish size, weight and overall health measures were taken from fish reared in aerated and non-aerated ponds.

Results and Discussion

The District purchased and installed an additional solar powered aerator to raise DO levels in two earthen ponds. With the additional aerator in place, DO readings were taken twice a day/ three days a week from January - December 2019 at 0800 and 1300h. Optimal dissolved oxygen levels for raising healthy, thriving mosquitofish should stabilize between 5-12 mg/L. Our findings showed that earthen pond DO tended to fall overnight due to the lack of sunlight and photosynthesis, so the solar aerators were run from 1700 – 1000h. In our earthen ponds, DO readings indicated that with a high biological load of fish, algae and other microflora at night respiration continues, but photosynthesis does not. In March 2019 the District stocked each test pond with 50 pounds of mosquitofish and continued to monitor pond health, as measured by turbidity, temperature and most importantly dissolved oxygen. The most important finding with our DO monitoring was that there were no major decreases in DO as observed in July for the aerated ponds. The solar aerator maintained DO levels from any major fluctuations (Figure 1). Fish harvesting was performed from June to late October and the overall yields in the aerated ponds were approximately

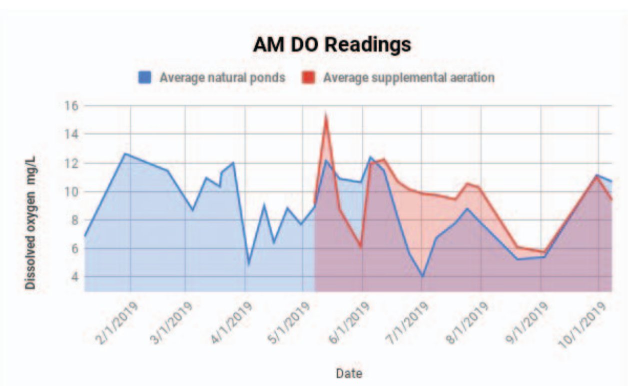

Figure 1.—Dissolved oxygen (DO) readings taken during morning (AM) for aerated and natural (non-aerated) ponds. No major dips in DO were seen in July for aerated ponds. The installed solar aerator prevented DO levels from major fluctuations.

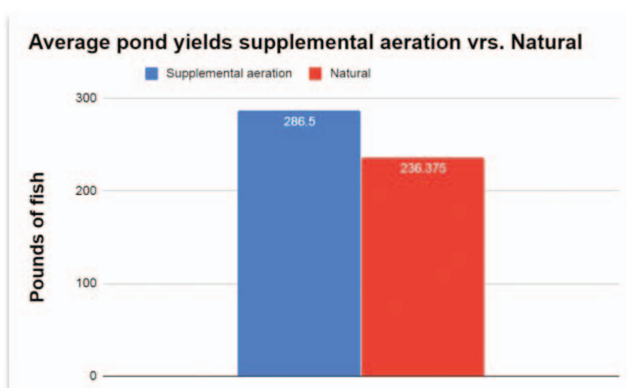

Figure 2.—Overall yields in average pounds of fish in aerated and non-aerated ponds. On average aerated ponds yielded approximately 50 pounds more mosquitofish.

50 pounds more mosquitofish than in the non-aerated ponds (Figure 2). Gravid female mosquitofish in the aerated ponds averaged 10% heavier and 4% longer (cm) compared to the non-aerated ponds (Table 1).

Conclusion

Ponds with this relatively inexpensive Keeton Industries Solaer® aerator SB-1-2 system proved to be healthier, produced larger mosquitofish, and increased yields overall.

Table 1.—Weight in grams and length in centimeters (cm) of gravid female and male mosquitofish in aerated and natural ponds.

	Aerated		Natural	
Average	Gravid Female	Male	Gravid Female	Male
Grams	2.32	1.52	2.08	1.27
	10% heavier	**12% heavier**		
CM	5.2	2.2	5	2.2
	4% larger	**Same**		

Effect of elevation on *Ixodes pacificus* nymph seasonality and abundance in the Sierra Nevada foothills

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Abstract

The western blacklegged tick, *Ixodes pacificus*, is a significant public health concern in California due to its capacity to transmit several tick-borne pathogens. The nymphal stage of the tick is the leading threat to public health due to its small size and typically higher infection rate of *Borrelia burgdorferi,* the causative agent of Lyme disease. Historically, large samples of nymphs have been difficult to collect in the Sierra Nevada foothills because they do not usually quest on vegetation along trails and are not as abundant as in some northwestern California habitats. The current study sought to increase our efficiency for collecting *I. pacificus* nymphs in the Sierra Nevada foothills by direct sampling of environmental substrates, such as rocks and logs, which have been shown to yield more nymphs than leaf litter. We flagged rocks and logs at 18 locations at elevations ranging from 330 to 3800 ft in 2018 and 2019. Collections at each location began in March and continued monthly until <1 nymph was collected. By increasing our collections of nymphs, we were able to compare their relative abundance and seasonal activity patterns at different elevations. Preliminary findings indicated that the prevalence and abundance of nymphs was similar on both rocks and logs, seasonal activity started later at higher elevations, and at elevations above 3000 ft nymphal ticks were active into August. This may indicate that the risk of encountering nymphs at higher elevations in California may extend throughout the summer months, rather than ending in late spring.

Automated tick disruption for detecting tick-borne pathogens

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Introduction

Tick-borne pathogens, such as the causative agents of tularemia, Lyme disease and Rocky Mountain spotted fever, can cause devastating life-threatening illness in humans (Wikel 2018). The San Diego County Vector Control Program (VCP) conducts surveillance for these pathogens in tick populations to protect the public health. The pathogen detection assays utilized by the VCP require efficient disruption of tick exoskeletons and isolation of the pathogen nucleic acids for polymerase chain reaction (PCR)-based diagnostic testing (Lejal et al. 2019). Previously, individual ticks were cut with a razor blade so that any internal pathogens could be detected. However, from 2015 to 2018, the number of ticks submitted to the laboratory for testing drastically increased (1,847 ticks tested in 2015 compared to 11,077 ticks tested in 2018) and the VCP was compelled to find a more efficient protocol to express internal pathogens from the ticks. We tested the TissueLyser II homogenization instrument (Qiagen) with a variety of milling media beads including ceramic beads, single stainless-steel beads as well as zirconium silicate beads and agate crystals, but none provided the desired effect. Freezing ticks in liquid nitrogen followed by grinding in a mortar and pestle was successful; however, it was labor intensive and was not efficient. Crowder et al. (2010) demonstrated the utility of using a combination of two sizes of high density yttria-stabilized zirconium oxide beads as a milling medium to homogenize ticks and a BioSpec Mini Bead Beater homogenizing machine. The larger sized beads served to crack open the tick exoskeleton while the smaller beads entered the crevices of the tick gut and mouthparts to expel the contents. The VCP investigated this technique for tick-borne pathogen testing to increase the efficiency in tick processing.

Methods

Up to ten ticks were homogenized within 2.0 mL screw-cap tubes (Sarstedt, Newton, NC) filled with 750 mg of 2.0 mm yttria-stabilized zirconium oxide beads, 150 mg of 0.1 mm yttria-stabilized zirconium oxide beads (MSE Supplies, Tucson, AZ), and 450 µL lysis buffer consisting of 425 µL of Qiagen ATL buffer and 25 µL proteinase K solution (Qiagen). The tubes were shaken in a Biospec Mini Bead Beater 24 (BioSpec, Bartlesville, OK) for 1 min and 30 sec, cooled for 1 min, and then the process was repeated twice. The samples then were centrifuged for 5 min at 15,000 rpm in a benchtop microcentrifuge and incubated at 65°C from 4 to 24 hours to allow enzymatic digestion of any nucleic acid inhibitors by proteinase K. After incubation, the tubes were centrifuged for 5 min at 15,000 rpm in a benchtop microcentrifuge. A 200 µL aliquot of the supernatant was transferred to a fresh 2.0 mL microcentrifuge tube. The samples then were loaded onto a QIAcube automated nucleic acid extraction instrument (Qiagen) using the DNeasy Blood & Tissue Kit (Qiagen) to isolate the DNA.

Results and Discussion

To determine the optimal bead 'beating time' needed to disrupt the exoskeletons of adult ticks, we performed a series of homogenizations, increasing the bead beating time by 30 sec intervals. After each of these intervals, the contents of the tube were poured out into a clear petri dish and visually examined under a light microscope. We found that the shortest period of homogenization time needed to disrupt the tick exoskeleton was 4 min and 30 sec. The BioSpec bead beater agitates microtubes in a 3D motion in contrast to a Tissue Lyser II which uses an axial back and forth shaking pattern. However, the BioSpec Bead Beater heated the microcentrifuge tubes when homogenization lasted 4 min and 30 sec. To mitigate any potential damage to the nucleic acids due to heating, we separated the homogenization time into three 1 min 30 sec intervals with 1 min cooling between intervals. To ensure that the nucleic acid was not being sheared during this process, all samples were spiked with known quantities of control DNA (UltraPure Salmon Sperm DNA solution, Invitrogen, Carlsbad, CA); similar quantities of DNA were recovered when samples homogenized at 4 min 30 sec compared to not homogenized.

Conclusions

The bead beating homogenization method developed by Crowder et al. (2010) was optimized and adapted by the VCP to increase processing capacity and efficiency in tick-borne pathogen testing. The first advantage of this protocol when compared to the razor blade method is that it saves time. Twenty four batches of ticks can be processed in 4 min and 30 sec with the BioSpec Bead Beater with no loss in sensitivity as compared to manually cutting individual ticks with a razor blade which takes approximately 1 hour. The newly adapted method also protects the user from potential injury caused by repetitive hand motion and eliminates the risk of working with sharp razor blades.

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Kissing Bugs and American Trypanosomiasis in California

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Abstract

Kissing bugs (Reduviidae) are blood-sucking ectoparasites and primary vectors of *Trypanosoma cruzi*, a parasitic protozoan that causes American Trypanosomiasis (Chagas disease) in humans. In California, *Triatoma protracta* is a widely-distributed species of kissing bug associated with *Neotoma* woodrats that serve as their preferred host and the trypanosome reservoir. However, locally-acquired human infections are extremely rare despite frequent human-bug encounters in suburban and rural areas. The California Department of Public Health, Vector-Borne Disease Section (CDPH-VBDS) receives inquiries from California residents regarding the risk of *T. cruzi* infection following exposure to kissing bugs in the home and collaborates with the US Centers for Disease Control and Prevention to test a subset of bugs submitted by the public. Between 2013 and 2019, 66 *T. protracta* (56 adults, 10 nymphs) and one *T. rubida* were submitted and tested for infection. Bugs originated from 17 counties and were collected primarily between May and September. Twenty-two (33%) *T. protracta* tested PCR positive for *T. cruzi* infection. Despite this relatively high infection prevalence, little evidence exists to suggest a significant risk of locally-acquired Chagas disease in California. The primary public health concern associated with kissing bugs in California is bite hypersensitivity; therefore, persons living in endemic areas should take measures to minimize bug attraction and entry into their homes. CDPH-VBDS has online resources available to the public and for public health professionals. (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/Conenose-Kissing-Bugs.aspx)

The Development and Use of a Duplex Real-Time PCR for the Detection of *Rickettsia typhi* and *Rickettsia felis* in Fleas Collected in Orange and Los Angeles Counties, California

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Abstract

The Orange County Mosquito and Vector Control District (OCMVCD) developed a duplex real-time PCR assay to test fleas for *Rickettsia typhi* and *Rickettsia felis*, the pathogenic bacteria responsible for human cases of flea-borne typhus. With the continuation of flea-borne typhus cases in Orange and Los Angeles Counties, OCMVCD was prompted to create a more efficient protocol to test for *R. typhi* and *R. felis* compared to singleplex real-time PCR assays currently in use for these *Rickettsia*. Fleas were collected from euthanized peridomestic host animals trapped by OCMVCD or pest control personnel, and opportunistically in the field from pet animals or human hosts during flea-borne typhus case investigations. All fleas were tested first using the singleplex real-time PCR and then retested with the duplex real-time PCR. A DNA sequence template synthesized to target the sequences of the primers and probes of both singleplex qPCR reactions, along with previously extracted *R. typhi* and *R. felis* genomic DNA from infected fleas, were used as positive controls to test for the efficiency of the duplex PCR. To determine the sensitivity of the assay, serial dilutions of our positive controls were used to evaluate the primers and probes in the duplex reactions. Of five species of fleas examined in this study, the cat flea, *Ctenocephalides felis*, was the flea that tested positive most frequently for *R. felis* (13.6%), whereas the dominant flea testing positive for *R. typhi* was the Oriental rat flea, *Xenopsylla cheopis* (8.9%). Our duplex real-time PCR assay was effective and specific for detecting *R. felis* or *R. typhi* in several different species of fleas.

Feral Pig Control in Alameda County

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Introduction

Feral pigs (*Sus scrofa*) were introduced to California by Spanish missionaries in the late 1700s with a subsequent introduction of Russian boar to Monterey in the 1920s (U.C.I.P.M. 2015). A survey conducted by John Fredrik in 1998 found feral pigs present in 45 of 58 California counties (Frederick 1998); however, today they are found in 56 of 58 counties (D.F.W. 2020). Feral pigs are competent vectors and reservoirs for a variety of pathogens which can affect the health of humans and livestock (Meng et al. 2009, U.C.I.P.M. 2015, West et al. 2009). Native wildlife species and local ecology also can be markedly impacted by pig populations (UCIPM 2015, West et al. 2009). Alameda County Vector Control Services District (District) operates a control program for pigs in conjunction with U.S.D.A. Wildlife Services. Each year the District receives calls from the public about property damage caused by feral pigs.

Methods

The District's feral pig program is request for services driven. After receiving a call, the District Biologist conducts a site inspection to verify the type and extent of the damage, suitability for trapping, and public health risks. During this inspection the Biologist provides advice to the reporting party based on the unique environmental factors present. In all human wildlife conflict cases where District Biologists provide guidance, the most important criteria for advice are: 1) access to food, 2) access to shelter (or similar resources; e.g., mud for thermoregulation), and 3) health and safety risks. If removal trapping is suitable, a referral is made to U.S.D.A. Wildlife Services who conduct a site assessment for trapping feasibility. Wildlife cameras are used to collect information on the age and sex of pigs comprising the sounder (group of pigs). Bait is used to assess the frequency of travel and to assist the cameras accurate detection of the number of animals. If bait acceptance occurs and the U.S.D.A. wildlife expert deems trapping suitable, a trap is set. Trapping requires that there is a point of contact on site who can check the trap every day, and inform U.S.D.A. Wildlife Services of captures. Additionally, it is essential that a firearm can be used safely for harvesting trapped animals. Traps used by our program are either "boss hog" cage style traps or corral traps. The modular nature of the corral traps allow them to be carried to sites not easily accessible by vehicle. Bait is fermented corn created by soaking dry corn in water in a sealed container (5-gallon bucket with an airtight lid, stirred daily). To be effective the corn must be fermented, not rotten. During pre-baiting, cages are wired open for a few days and monitored using a game camera, with bait broadcast in front of the cage and piled behind the trip line, usually buried under leaves or sticks gathered onsite. In places where deer are of concern, a portion of the entrance may be blocked by affixing a log or barrier over the entrance. Local police are notified in advance by the U.S.D.A. and a depredation permit obtained from the Department of Fish and Wildlife (D.F.W.). Trapped animals are harvested using a .22-caliber rifle, so having the trap placed where a firearm can be used safely is essential.

Results and Discussion

In 2019 Alameda County Vector Control Services District eliminated over 30 pigs from the County. Trapping operations occurred in Castro Valley, Dublin, and Fremont. Blood was collected on Nobuto strips from some trapped animals and was submitted to C.D.P.H. for plague testing. Ectoparasites were collected from trapped animals when District Biologists were available. At this time, we are not aware of any plague positives in blood obtained from the pigs. The ectoparasite composition was primarily *Pulex spp*. fleas, *Dermacenter spp*. ticks and lice.

Conclusion

As property damage from feral pigs continues to impact Alameda County residents, the District will maintain these trapping operations in conjunction with U.S.D.A. Wildlife Services. When harvested as part of wildlife management program, feral pigs can serve as important sentinel species for disease surveillance and exposure of anticoagulant rodenticide in wildlife (Berny 2020).

Acknowledgements

Thank you to Lucia Hui, Robert Gay, Bruce Kirkpatrick, Paul Cooper, Natalia Fedorova and the staff at Alameda County Vector Control. This program would not be possible without partnership U.S.D.A. Wildlife Services, California Department of Public Health, Alameda County

Animal Services, and local law enforcement. Thank you to Roger A. Baldwin and Niamh Quinn at U.C.A.N.R. Cooperative Extension for always answering my questions and to Dr. Reisen for his patient help with editing.

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Large-scale suppression of *Aedes aegypti* in California by release of *Wolbachia*-infected male mosquitoes

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Abstract

Aedes aegypti presents an immense burden on human health globally and is a major nuisance in many areas, particularly California, where there is currently no virus transmission by this mosquito. Traditional mosquito abatement techniques have not been sufficient to control this invasive mosquito, underscoring the need for new, cost-effective technologies such as the Incompatible (or Sterile) Insect Technique that can be deployed across large areas. We have developed new automated processes to rear and release millions of competitive, male-only *Wolbachia*-infected male mosquitoes. Building on highly successful suppression in three Fresno County, California communities in 2018, we expanded releases in 2019 while also varying the target 'overflooding' ratio of released-to-wild males. Due to permitting delays, daily releases did not begin until mid-June and lasted until the end of the mosquito season in Fresno County. We monitored wild *Ae. aegypti* in the release areas as well as similar non-release neighborhoods and found that 1) the mosquito population increased at a much slower rate in areas where mosquitoes were previously released in 2018 relative to areas that did not have previous releases, and 2) in areas where the target overflooding ratio was highest, the number of female mosquitoes was more than 75% lower in the release areas compared to non-release areas at peak mosquito season. Our results demonstrated that significant suppression of *Ae. aegypti* using SIT/IIT is feasible when mosquito densities are already high, while also highlighting the advantage of starting SIT programs when mosquito densities are lowest.

Aedes aegypti, West Nile virus and SIT: The Perfect Storm

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Abstract

From 2016-2019 the Consolidated Mosquito Abatement District (CMAD) has operated under a USEPA experimental use permit (EUP) to study the use of *Wolbachia* infected male *Aedes aegypti* mosquitoes for control in selected urban areas of Fresno County, California. Each year of study following the first year of releases in 2016 allowed for change and modification based on lessons learned from the previous year. In 2019 the District found itself facing a totally new set of challenges and setbacks in mosquito control. The District recorded record high *Ae. aegypti* abundance, a record number of calls for service and our second highest year of West Nile virus activity to date, while trying to support a robust sterile insect technique (SIT) program in select neighborhoods. This presentation summarizes these challenges and discusses how the CMAD worked to manage and minimize negative impacts to ongoing efforts with the SIT program, Debug Fresno.

Measuring Impact of School Outreach to Change Mosquito Prevention Behaviors

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Introduction

Educational outreach to schools is a vital component to the County of San Diego Vector Control Program's (VCP) integrated vector management strategy and its efforts to inform the public about mosquito prevention. Determining whether the information about preventing mosquito production at home in these presentations is being adopted by students and their parents at home is important to measuring the effectiveness of the program. We designed a Vector Mission School Program to quantitatively measure the impact of our school outreach program on changing behavior toward mosquito prevention. The project involved a two-part workbook series that educates students and collects data about potential mosquito breeding sources and changes in prevention behavior in households.

Methods

The VCP Outreach group's school program supports the Next Generation Science Standards for California public schools and includes a presentation and interactive activities. The presentation teaches mosquito biology, diseases, and prevention practices and the activities include examining the mosquito lifecycle under microscopes and completing activity books at home (Figure 1).

Figure 1.—Activity books with microscope and program materials.

The activity books collect data from students before and after program implementation. Activity Book 1 is given before the outreach team visits the class and has pictures of 10 potential breeding sources for students to mark off if they have these at home. Activity Book 2 is given after the presentation is completed. The students are instructed to go back to the sources they found in Activity Book 1, check if they have standing water, and either dump out the water or tell an adult.

The Vector Mission School Program is completed in three steps. The first step is contacting the teacher via email, providing the lesson plan, setting a date for the classroom presentation, and delivering Activity Book 1 to the school. The second step is the classroom visit. The outreach team conducts their presentation, collects the completed Activity Book 1, and distributes Activity Book 2. The teacher emails the outreach team when the completed Activity Book 2s are ready to be picked up. The third and final step is collecting Activity Book 2 and distributing student rewards and certificates for their participation.

Results

The Vector Missions School Program was piloted in fourth and fifth grades. Based on previous classroom observations by the outreach team, fourth and fifth graders tended to be more responsive and cognitively developed to retain and recall information. The outreach team conducted the Vector Missions School Program at 4 schools, giving 9 presentations and interacting with 259 students. Two hundred and seven students (80%) completed all parts of the Program.

Figure 2 illustrates the percentage of student households that had potential larval habitats. Flower vases, outside toys, and potted plants with plates were among the most prevalent sources found. After identifying potential larval sources in the community, the next step was to determine if there was standing water present and if action was taken to eliminate the source.

Two hundred two students (78%) either dumped out water or told an adult there was water found in a source they were unable to dump out, specifically yard drains and fountains (Figure 3). The top 3 sources containing water were flower vases, outdoor potted plants with plates, and

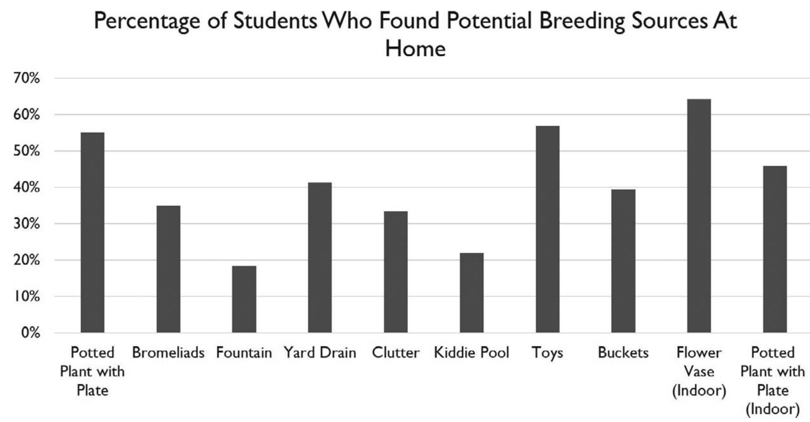

Figure 2.—The percentage of students who found each potential larval source at home.

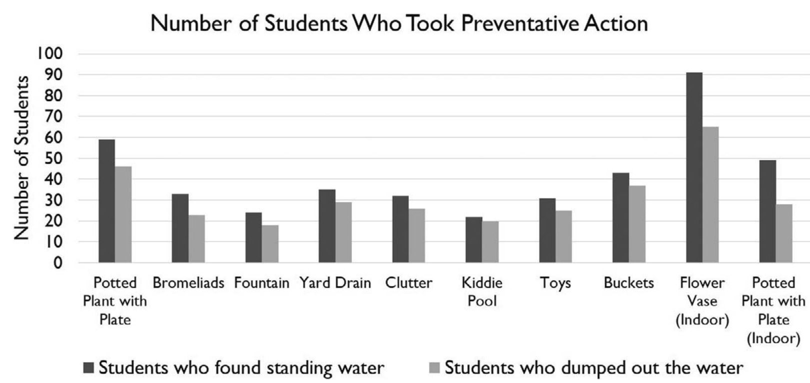

Figure 3.—Number of students who found and took preventative action with each type of larval habitat containing water.

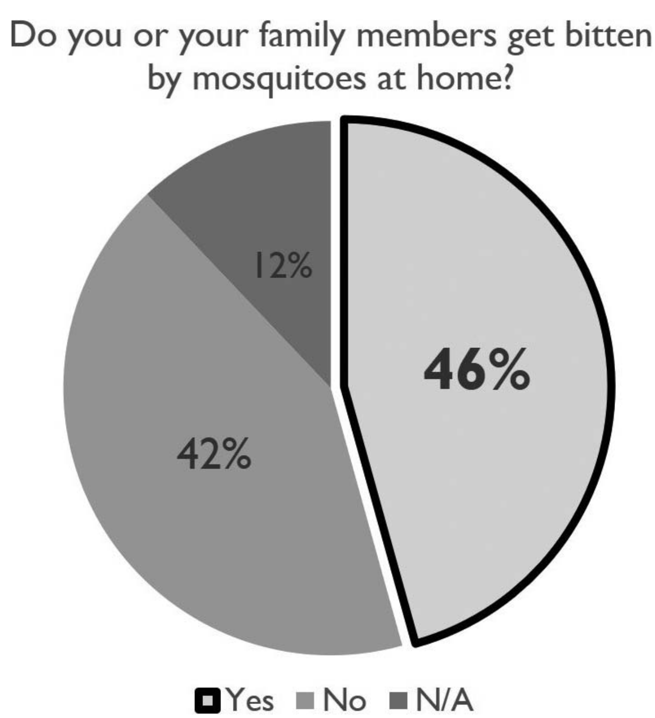

Figure 4.—Response to question (n = 116): "Do you or your family members get bitten by mosquitoes at home?"

indoor potted plants. Overall, water was dumped out in 71%of flower vases that contained water,78% of outdoor potted plants with plates, and 57% of indoor potted plants.

A short 4 question parent survey was included at the end of Activity Book 2. The first question asked if the family was bitten by mosquitoes at home, and the second question asked if it was known before the student began this assignment that mosquitoes develop in standing water, allowing us to measure the parents' initial knowledge of mosquitoes. Forty-six percent of parents responded that they were bitten at home (Figure 4) and 36% answered they didn't know mosquitoes developed in standing water (Figure 5). These results indicated that many parents may not understand the reason they are getting bitten at home and helped the outreach program to focus its educational messaging.

Question 3 asked if the parents intended to continue to dump out standing water. Seventy-two percent of parents said they will continue to dump out standing water (Figure 6), which equates to 184 homes actively working to not contribute to the mosquito population and that may not require a vector technician to do a mosquito inspection.

The fourth question instructed the parents to fill in the blank with how they usually prevent mosquitoes. The top two responses were aligned with our Fight the Bite message, "wear repellent" and "dump out standing water." A common response observed was to use bleach to control

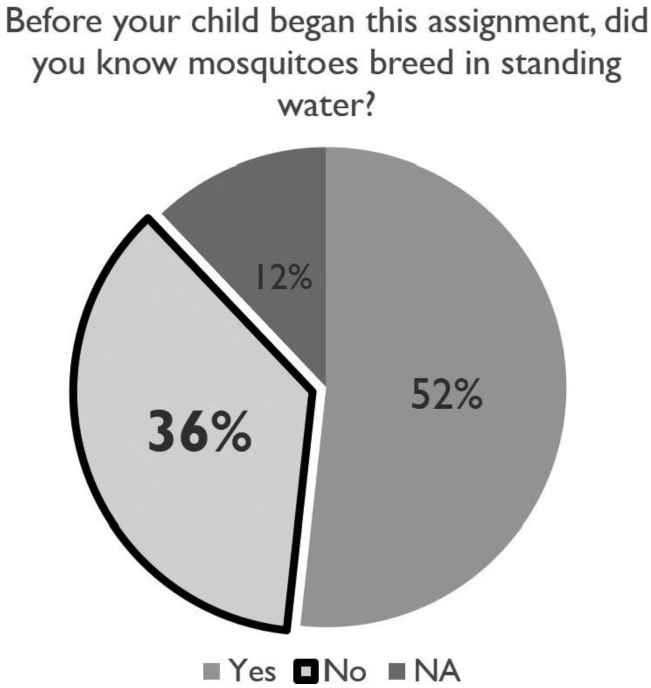

Figure 5.—Response to question (n = 116): "Before your child began this assignment, did you know mosquitoes breed in standing water?"

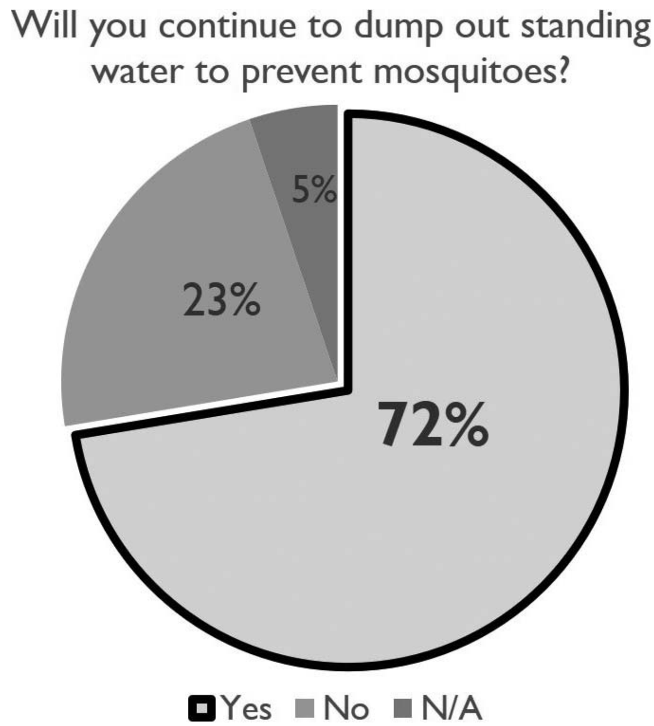

Figure 6.—Response to question (n = 116): "Will you continue to dump out standing water to prevent mosquitoes?"

mosquitoes, which revealed possible misconceptions that could be addressed when devising outreach messages in the future.

Conclusion

The program was successful in achieving its primary goal which was to measure the change in behavior towards mosquito prevention following the outreach school program. Approximately 2 hrs was invested per class, reaching about 777 people. The results obtained enabled a quantitative evaluation of VCP's educational and outreach program.

Acknowledgements

We would like to thank Allison Bray and Anna Kremen for their help implementing this project.

Public Outreach Response to Detection of Invasive Mosquitoes in Sacramento County

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Introduction

The invasive mosquito *Aedes aegypti* was first detected in Sacramento County in August of 2019. Although the District had a Communications and Outreach plan in place, the new and unexpected detection demanded that the plan activated at an accelerated pace. This presentation provides an overview of the various public outreach activities that were put into place to alert, inform and educate residents in the detection area about this invasive mosquito.

Methods

Upon the detection of *Aedes aegypti*, a press release was sent to the media outlining details of the detection and the District's immediate initiation of door-to-door - inspections within the affected neighborhood in Citrus Heights. As a result of the press release, a press event was planned near the original detection site the day after the detection was announced. The press event was carefully planned to include a demonstration of a barrier treatment by a field technician at the home of a local resident. This event resulted in intense media coverage that helped to spread prevention messages to District residents. Another successful outreach strategy included attending a variety of local community events to inform residents in specific neighborhoods where door-to-door inspections and surveillance were being conducted. It was important to share information regarding all District activities, encourage residents to report day biting mosquito activity and educate them on mosquito prevention tactics they could take to ensure they were not producing mosquitoes at home. In total, the District attended 5 events in less than six weeks. A variety of materials were produced to be distributed by District staff, including an invasive species door hanger brochure, a brochure, and speaking points for staff when addressing the public, and a postcard was sent out to all homes within the detection area. Another important outreach strategy was immediately notifying local elected officials of the detection of invasive mosquitoes in Citrus Heights via an email when mosquitoes were initially detected, followed by periodic updates, giving a presentation at a Citrus Heights city council meeting, and having face-to-face meetings to keep them informed.

Results and Discussion

The communication and outreach strategies proved to be very useful in ensuring that the public was educated about the detection of invasive mosquitoes in their community. Some of the challenges that the District faced while trying to roll out an en effective communications plan included rapidly changing and evolving information. Therefore it was difficult to plan in advance. In addition, implementing the outreach plan required that District staff to conduct these activities and in addition to the many District activities occurring at the same time.

Conclusion

Invasive *Ae. aegypti* mosquitoes were detected in Sacramento County in August of 2019. An effective outreach and communications plan was quickly set in motion to ensure that residents were informed of the detection and all District activities. Moving forward, the District will continue outreach efforts where invasive mosquitoes are detected, will implement and expand outreach activities as necessary, continue exploring innovative control techniques and develop more materials needed to ensure the effectiveness of the community outreach plan.

Acknowledgements

The Sacramento-Yolo Mosquito and Vector Control District staff for their time in participating in events to help inform District residents.

Geotargeting social media outreach using Nextdoor

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Introduction

Nextdoor is a social media website and mobile app that connects people to others in their neighborhood and surrounding areas. Neighborhoods are, as described by Nextdoor, "a private environment designed just for you and your neighbors" (Nextdoor 2020). The boundaries of neighborhoods are defined by the residents of those neighborhoods and can include anywhere from 100 to 3,000 households. Residents are required to verify their identity and address to join the site. In San Diego, over 700,000 households have a Nextdoor account, which is about 36% of all households in San Diego County. Originally, Nextdoor was designed to be used only by individual residents, but Nextdoor added a public agency partner feature to allow public agencies to share information with those they serve.

Public agency partner accounts are free for certain organizations, including local governments and agencies that provide emergency services. Other regional authorities may gain access to a paid service provider account by paying a fee that is based on the population of their jurisdiction. When posting to Nextdoor, public agency partners can make posts visible to their entire jurisdiction, individual cities/areas, or individual neighborhoods. Users do not need to 'opt-in' to posts from public agencies and are automatically subscribed to receive messages from all agencies that serve their area. Public agency accounts can only post to their jurisdiction; they cannot see, search for, or respond to posts made by residents except for comments on the public agency's own posts (Nextdoor 2019).

Currently, few vector control districts have a presence on Nextdoor, but conversations about mosquitoes and other vectors are already happening on Nextdoor with or without them. People share problems they are having, such as a high number of mosquito bites, or an area they believe may be 'breeding' mosquitoes. In areas experiencing mosquito problems, information about how to combat the mosquitoes often comes from misinformed residents and may be incorrect. For example, resident-provided mosquito-prevention suggestions on one post included taking B12 vitamins, wearing mosquito repellent wristbands, and planting mosquito repelling plants. Because of the limitations on public agency accounts, vector control districts cannot respond directly to misinformation spread by residents but can provide correct information to Nextdoor users.

Methods

The San Diego VCP uses Nextdoor by collaborating with the County Communications Office to post to the County of San Diego public agency partner account.

Results and Discussion

Because Nextdoor posts can be targeted to specific neighborhoods or areas, customized messages particular to the location can be delivered much more specifically than less geo-targeted social media such as Facebook or Twitter. For example, VCP made a post for residents in an area of a city where larval production from sump pumps was common but was rare in the rest of the county. Because sump pumps are not a common feature in most San Diego homes, a regionwide outreach effort would not have been appropriate. In addition, sending a mailer to residents in this area was cost prohibitive. Fortunately, one affected resident had given feedback that members of their community primarily shared information via social media including Nextdoor, so we posted information about sump pumps to their Nextdoor neighborhoods. Out of 8,500 total households in Coronado, a post to Nextdoor potentially reached almost 2,300 households, just over 25% of the households in the city. The cost of mailing letters to the same number of households would have been greater than $1,400 including printing costs, postage, envelopes, and stuffing and addressing each mailer. Using the San Diego County public agency partner account, this post was free – making it a cost-effective form of neighborhood outreach. Districts considering their own Nextdoor accounts should evaluate cost effectiveness by comparing the cost of account access to the cost of reaching a similar number of households through physical mailings or other geo-specific outreach methods.

Nextdoor posts also can provide information tailored to what mosquito species the neighborhood is having issues with – an area experiencing high levels of salt marsh mosquitoes may need more information about ongoing larvicide efforts and repellent advice, whereas an area experiencing invasive *Aedes* may need information targeted to finding and dumping out cryptic water sources. Individual characteristics of neighborhoods, such as the age of residents, or preferred language of residents can be incorporated into the messaging as well.

Because the geographical reach of a Nextdoor post is known, the effects of the post can be measured using program data (such as service calls) from within the same area. Another Nextdoor post made by the VCP was delivered to an area that was having issues with *Aedes* mosquitoes. Technicians reported a high volume of service calls from the area that required the same mosquito production prevention information to be repeated to many neighbors. They requested that the outreach team assist in sharing information to this neighborhood in a way that would inform the residents but also decrease the service requests to the technicians. The VCP posted a Nextdoor message about cryptic breeding sources, locally common larval sources (rain barrels), and repellent use to the affected neighborhoods. In the six weeks following this post the average number of mosquito-related service calls increased 70% county wide, but decreased 23% within the neighborhoods targeted by the post (when compared to the six weeks before making the post). Certainly, there were other factors involved, but the trend is interesting enough to continue using the process to measure the effectiveness of Nextdoor outreach.

Potential future uses of Nextdoor for our program may include proactive posts to areas that have had problems with *Aedes* mosquitoes in previous seasons. Comparison of trap counts within a neighborhood before and after Nextdoor messaging may reveal if it can be associated with decreasing invasive *Aedes* mosquitoes. Another potential use for Nextdoor is to accompany targeted mailing to areas where West Nile virus has been detected or to accompany announcements of planned adulticide applications to increase awareness of these activities.

Conclusions

The benefits of using Nextdoor for vector control agencies include increased reach to social media users, a presence on a new social media platform where information about vectors is already being shared, the potential for highly customized messaging, and the cost effectiveness of Nextdoor posts over physical mailings. It also provides the potential to measure the effectiveness of social media outreach. Districts considering using Nextdoor should compare cost effectiveness compared to traditional outreach methods and consider reaching out to city or county social media teams to see if they can cooperate on mosquito related social media posts.

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Media campaign strategies for maximizing return on investment

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Introduction

Vector control agencies use media campaigns to help achieve awareness, education, and behavioral changes by the public. Media platforms include TV, radio, posters and billboards, as well as other innovative media such as vehicle wraps and digital formats e.g. web search engines, emails, web banners, social media, mobile devices and connected TV advertisements. Evaluating the effectiveness and return on investment (ROI) of each of these platforms depends on the goals of the media campaign and is important for creating fiscally effective campaigns

Methods

Costs, impressions, and website traffic from the 2015-2019 County of San Diego *Fight the Bite* media campaigns were evaluated. All media campaigns were centered on the Fight the Bite messaging of *Prevent, Protect and Report*. Impressions were measured and provided by the companies that distributed the campaign content which included TV and radio stations, Clear Channel and American Outdoor, Facebook and YouTube, as well as other digital services. Engagement was assessed by tracking "mouse clicks" on digital media and the number of visits to the County's SDFighttheBite.com website as measured by Adobe Analytics. Engagement also was measured by the number of times the 15 second *Fight the Bite* educational videos, displayed via pre-roll video and connected TV advertisements, were viewed to completion. Because the videos contained much of the same information provided on SDFighttheBite.com, it was assumed that viewers who completed the videos participated in a similar "engagement" with educational content as if they had navigated to the website. The return on investment was calculated as the amount of money spent on the media format divided by the number of impressions (cost per impression) or divided by the number of website visits or video completions (cost per engagement).

Results and Discussion

The cost per impression ranged from 0.003 to 0.009 cents per view of media content. Overall, 2016 had the highest cost per impression, likely because the campaign that year did not include advertisements placed on outdoorplatforms such as billboards or posters that, have a low cost/impression. In 2019, social media and outdoor advertising achieved the lowest cost per view of $0.002 and $0.003 per view, respectively. The highest cost per view was connected TV at $0.036 per view. However, cost per engagement showed a different trend. Web videos ($0.02), connected TV ($0.04) and social media ($0.58) demonstrated the lowest cost per engagement (Fig. 1).

Daily measurement of SDFighttheBite.com website traffic enabled comparisons between different media campaign inputs. The April 2019 media campaign increased website traffic from approximately 25 visits per day in the previous month to 150-425 visits per day during the campaign (Fig. 2). The website during the April 2019 campaign had 3-8.5 times as many views as during the spring 2018 media campaign. This increase in website traffic was due to a greater focus and budget allocation towards digital advertisements designed to drive visits to SDFightTheBite.com. This data also was examined to look for associations between media launches during the campaign and spikes in website traffic to determine the efficacy of each media platform. Campaign inputs such as outdoor advertising, digital advertising, and email distributions were followed by increases in website visits (Fig. 2). The demographics reached by digital content and their response to different digital formats also were measured and used to redirect funds mid-campaign to media content

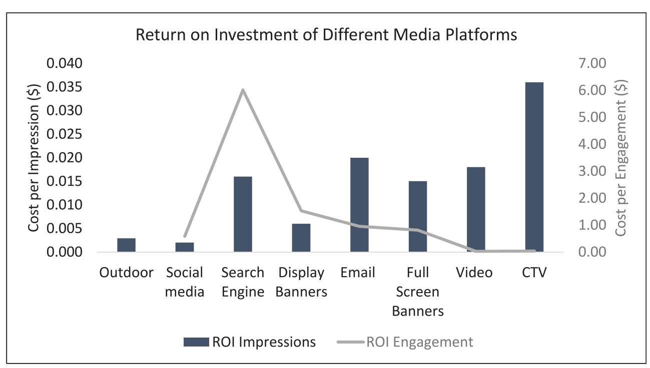

Figure 1.—Cost per view of media content (impression) and cost per website visit or video completion (engagement) for each media in the County of San Diego Fall 2019 Fight the Bitecampaign. ROI is return on investment.

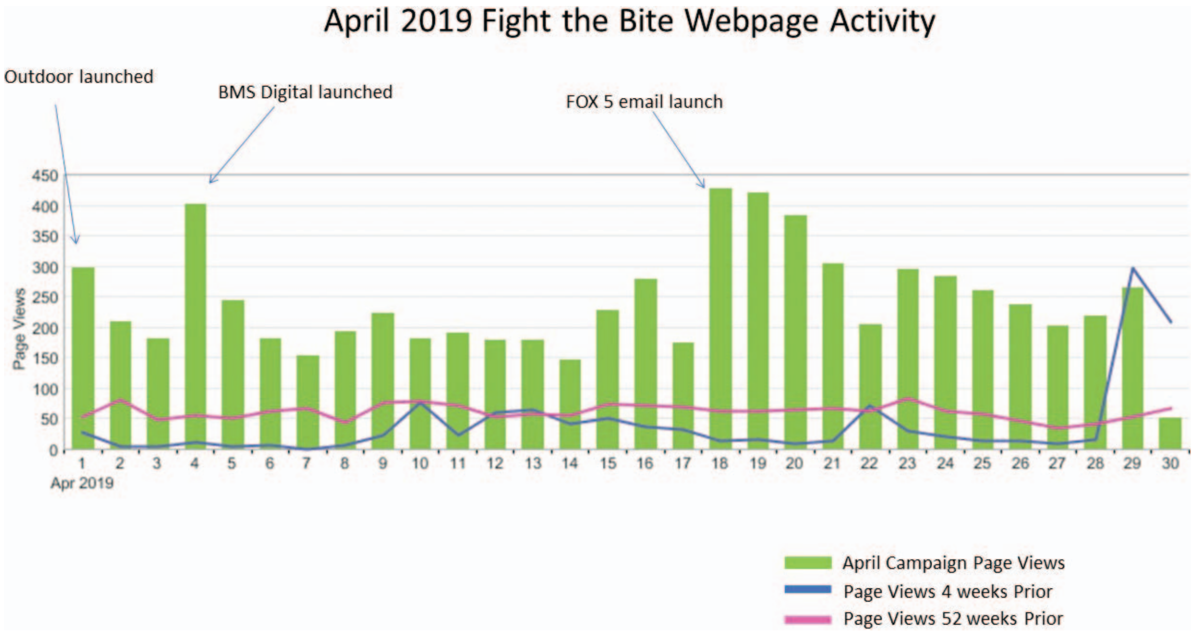

Figure 2.—Visits to the SDFighttheBite.com website during the County of San Diego Spring 2019 Fight the Bite media campaign. The blue line shows the number of website visits 4 weeks prior to the campaign. The pink line shows the number of visits 52 weeks prior during the 2018 campaign. Data compared by day for April 2019.

and formats that achieved better responses within the targeted demographics (data not shown).

Conclusions

Public response to media campaigns should be measured and evaluated to determine how to allocate funds to attain campaign goals and maximize return on investment. Diversified media strategies enable campaigns to achieve diverse goals and to focus on successful platforms, formats, and content.

Acknowledgements

We thank Anna Kremen for her careful review and editing of this manuscript.

Community Liaisons: Building Trust, Collaboration, and Community Leaders for Vector Control

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INTRODUCTION

The Greater Los Angeles County Vector Control District (GLACVCD/District) is a public health agency formed in 1952 under the authority of the California State Health and Safety Code with a mission to reduce populations of vectors below nuisance levels and prevent human infection associated with mosquito-transmitted pathogens. GLACVCD is the largest of five vector control districts serving the County of Los Angeles with a service area over 1,300 square miles, encompassing 35 cities and unincorporated areas of the county, and nearly six million residents.

With the continuous spread of *Aedes* mosquitoes throughout the County, the District needed to bolster its presence in the community and increase outreach to maintain partnerships, expand education and awareness, and build trust. Because of the geographical challenge of the District and its large and diverse demographics, the Department of Community Affairs added three additional positions in 2019 to improve its public outreach, education, and presence.

METHOD

GLACVCD's robust outreach and education program has included comprehensive paid media campaigns, in-classroom curricula, and community presentations and events. Despite these efforts, the continued spread of *Aedes* mosquitoes throughout the District's service area posed a challenge in creating behavioral change among residents and analyzing the efficacy of current outreach methods. In 2019, the District added three community liaisons positions to work extensively within the community to mobilize, educate, empower residents, and create community leaders for vector control. The District's community liaison positions were each assigned to one of three zones: 1) South East Los Angeles County, 2) Los Angeles City proper and 3) San Fernando Valley and parts of North Los Angeles County.

In the initial months, the community liaisons completed extensive research to identify key factors about their assigned zones, such as demographics, socioeconomics, community organizations, faith leaders, environmental groups, business chambers and other important stakeholders. At the height of the mosquito season, the community liaisons were able to attend outreach events, provide presentations and conduct door-to-door efforts automatically increasing our presence, reach, and trust among local leaders.

RESULTS AND DISCUSSION

Community liaisons are new positions to the field of vector control, but commonly are used in public agencies throughout the County. In less than a year, their addition to the District's community affairs department has helped surpass the reach of GLACVCD within its service area (Figure 1). The community affairs department attended more community events in 2019 than in previous years, and these efforts contacted 22,000 residents, more than any other year.

These interactions were meaningful and helped to establish relationships within the communities of the District. However, consistent interactions with these communities are essential to creating behavioral changes necessary to decreasing *Aedes* populations and the risk of mosquito-transmitted pathogens. In the coming years, the community liaisons will work on building and maintaining relationships with established community organizations

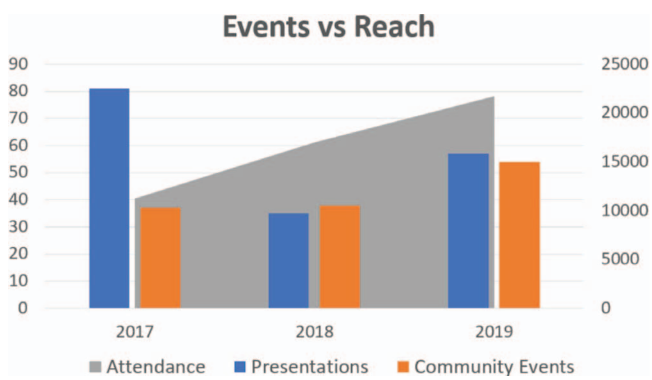

Figure 1.—The number of presentations and events the District participated in from 2017 through 2019The gray graph shows the gradual increase in number of people reached through these events. Despite attending less events in 2019 than in previous years, the District reached more residents than in previous years and in less time due to the strategic efforts of the community liaisons.

that have gained the trust of residents. By creating partnerships with these organizations, the District will not only become a trusted source for information and services related to mosquito control, it will also create community leaders that could influence other neighborhood residents to make behavioral changes. This same approach will be applied to other stakeholder groups such as neighborhood councils, parent groups, homeowner associations and other grass root-level organizations. Additionally, to measure the efficacy of the community liaisons, their efforts will be measured over time through the use of surveys to track whether their presence at community events, presentations, and meetings are truly having an impact on mosquito abundance.

CONCLUSIONS

In addition to the District's traditional media and outreach campaigns, community liaisons have proven to be effective in reaching more residents than previous methods. Furthermore, their efforts can be measured qualitatively, something that is not as easy to analyze in paid media campaigns and social media outreach strategies. Further research into how effective their activities are will help the District analyze ways to improve its outreach programs.

ACKNOWLEDGEMENTS

Special thanks to all GLACVCD staff and to General Manager Truc Dever for the opportunity to initially launch and oversee this program.

#GovernmentInfluencer, Using social media, videos and viral trends to increase engagement as a government agency

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Abstract

Social media has become a critical way to communicate with others. The use of video, photos and engaging social media posts is key to increasing engagement and awareness with residents. By using technology, trending internet challenges, and integrating a challenge in the Orange County Mosquito and Vector District's summer campaign, the District was able to enhance the 2019 MissionTipNToss Campaign to reach more people. This presentation offers recommendations on how districts can use existing partnerships and technology to enhance social media and community participation.

You’re OUT (reach)! Working with minor league baseball to increase District presence

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Abstract

Vector Control Districts are always looking for novel outreach opportunities that provide the largest audience in the most economical way. In the pursuit of this, the West Valley Mosquito and Vector Control District entered into advertising agreement with the Rancho Cucamonga Quakes, a local minor league baseball team that is associated with the Dodgers. For an investment of $3,700.00 (not including the cost of giveaway items), the campaign included a web presence, branding on their newsletters, an informational booth at the home game of our choosing, and an on-field promotion – a game played by fans during inning switches with giveaways provided by the District. With an estimated 70 home games played and an average attendance of 2,450 per game and a minimum of 45 email newsletters with approximately 5,500 subscribers, the District’s message was seen by a large number of residents at a very low cost per person. We believe this method of outreach was a novel, economical and wide-reaching campaign that brought recognition and interest in vector control to a large portion of our residents.

Pairing individual resistance phenotype information with SNP data in *Aedes aegypti* from California

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Abstract

Since detection in 2013, *Aedes aegypti* have become a widespread pest in California. Pyrethroid resistance in these mosquitoes has been a significant challenge to control efforts. This resistance has largely been attributed to mutations in the voltage gated sodium channels (VGSC), the pyrethroid site of action. To investigate this mechanism, we sampled individuals from four locations in central California and the Greater Los Angeles area. Individuals from each population were subjected to a pesticide exposure assay to determine knock down time. After extracting each individual's DNA, we used the iPLEX MassARRAY System to analyze a total of 36 single nucleotide polymorphisms (SNPs), 5 of which represent non-synonymous mutations in the coding region of the VGSC gene. Three of these are associated with resistance in the literature, and two are new and untested. We discovered that the SNP profile of mosquitoes originating from the Greater Los Angeles area were significantly different from those originating in the Central Valley, consistent with the previous findings supporting multiple introductions of *Ae. aegypti* to California. The use of the iPLEX MassARRAY system is a powerful tool for rapid detection of large arrays of SNPs and could be applied to monitor population dynamics and resistance alleles throughout California. We will continue to explore the biology of these mosquitoes to understand how environmental, biochemical and genetic factors influence resistance phenotypes. This work will aim to identify new resistance mechanisms and the development of new techniques to predict resistance in field populations.

Resistance Testing and Mosquito Management with Pyrethroids

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Introduction

Extensive use of pest control products can exert selection pressure on target populations and can drive the development of resistance, threatening the efficacy of the already limited number of public health pesticides. The Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) maintains a resistance management program which includes the use of field cage assessments of adulticide applications as well as bottle bioassays of mosquito populations within its boundaries. However, although growing resistance to pyrethroids has been documented using the bottle bioassay, results of field cage tests with formulated products were at times very different. Adding a synergist to the bottle bioassay has historically been used to overcome resistance and elucidate the mechanism involved. The question of whether or not this approach could also provide a better estimate of the actual resistance profile of wild mosquito populations provided the impetus for this study.

Materials and Methods

Bottle bioassays of adult mosquitoes were conducted at SYMVCD following the protocols outlined in the CDC Guideline for Evaluating Insecticide Resistance in Vectors Using the CDC Bottle Bioassay starting with recommended bottle dosages stated in Procedure for Calculating Time-Mortality Curves using a Bottle Bioassay (Peterson, J. 2007). Final dosages for some active ingredients used by SYMVCD previously had been fine-tuned by generating the diagnostic dose and diagnostic time appropriate for our local mosquito species.

All chemical standards utilized in bottle bioassays were obtained from Chem Service Inc., West Chester, PA. Mosquito populations were exposed at the dose of 43 ug/bottle when the permethrin chemical standard was tested. In the bioassay using both a permethrin chemical standard and a piperonyl butoxide (PBO) chemical standard, bottles were first dosed with permethrin at 43ug/bottle then PBO at 400 ug/bottle. The deltamethrin chemical standard was used at the locally optimized dose of 22ug/bottle rather than the 30 ug/bottle sample reference dose suggested by CDC for the continental United States. When comparative bottle bioassays were performed using the formulated product DeltAGard against a deltamethrin chemical standard, a 1 ug/ul working solution of each product was prepared to ensure the same dosages were used.

Field evaluation of the adulticides Pyronyl Oil Concentrate #525 (Central Life Sciences, Schaumburg, IL.; 5% pyrethrins, 25% piperonyl butoxide (PBO) and DeltAGard (Bayer, Pittsburg, PA.; 2% deltamethrin, 98% other ingredients) applied by truck were done by placing adult mosquito sentinel cages (Townzen and Natvig 1973) in an array of 4 cages on a single stake placed perpendicular to the spray path. A total of four transects each containing five of these 4 cage arrays placed at 50-foot increments were used. Each array was composed of cages containing 20-25 of the following mosquitoes: susceptible laboratory colony *Culex quinquefasciatus* (CQ1), resistant *Cx. pipiens* (Woodland colony pipiens, WCP), susceptible laboratory colony *Culex tarsalis* (Kern National Wildlife Refuge strain, KNWR) and resistant field-collected *Cx tarsalis*. Pyronyl #525 was applied at the rate of 0.8 fl oz/acre and DeltAGard was applied at 0.67 fl oz/acre using ULV Foggers (London Foggers, Minneapolis, MN; model # XKE). Trucks were driven at 10 mph.

Results

Post-spray sentinel cage evaluations of Pyronyl Oil Concentrate #525 showed $\geq$97% mortality in susceptible populations of both mosquito species, but mortality in wild populations was variable (Fig. 1). At the point closest to the spray path, field-collected populations of *Cx. tarsalis* and *Cx. pipiens* had 83% and 80% mortality, respectively. Even with ideal application conditions such as an inversion, appropriate wind speed and droplet size, other factors such as pesticide resistance, vegetative cover or other physical barriers and species of mosquito can cause these differences in mortality. Bottle bioassays can be used as a tool to separate resistance from the other factors that may negatively affect spray efficacy. Figure 2 shows the results of bottle bioassays using a permethrin standard against the same mosquito populations used in the sentinel cages. Mortality rates in the bottle bioassay for susceptible populations were similar to those observed in field cages, but mortality for the resistant field mosquito populations remained around 20% for both field-collected species. The bottle bioassay was repeated against the same mosquito

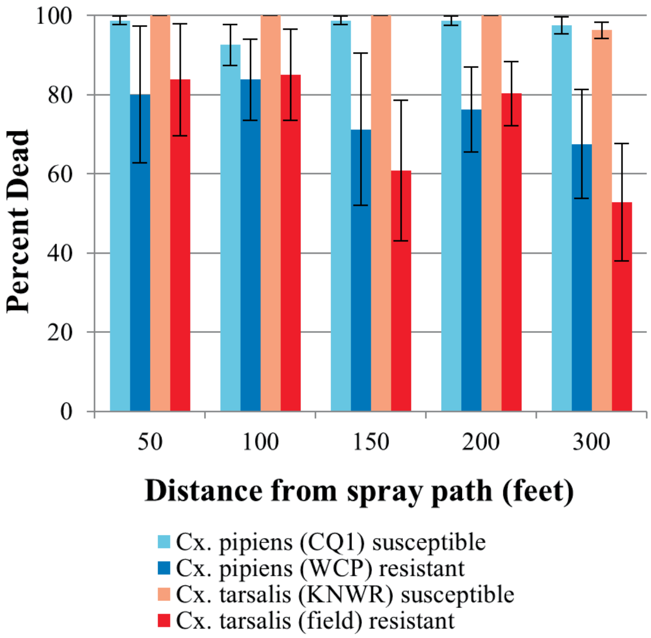

Figure 1.—Mortality in adult mosquitoes from 4 groups at 12 h post application in sentinel cages from a 2019 field evaluation of the adulticide Pyronyl Oil Concentrate #525 in Sacramento County, CA.

species with the addition of PBO (Fig. 3). The results of this second bottle bioassay using permethrin plus the synergist found in Pyronyl Oil Concentrate #525 yielded results that were more closely aligned with the performance of the formulated product in the field.

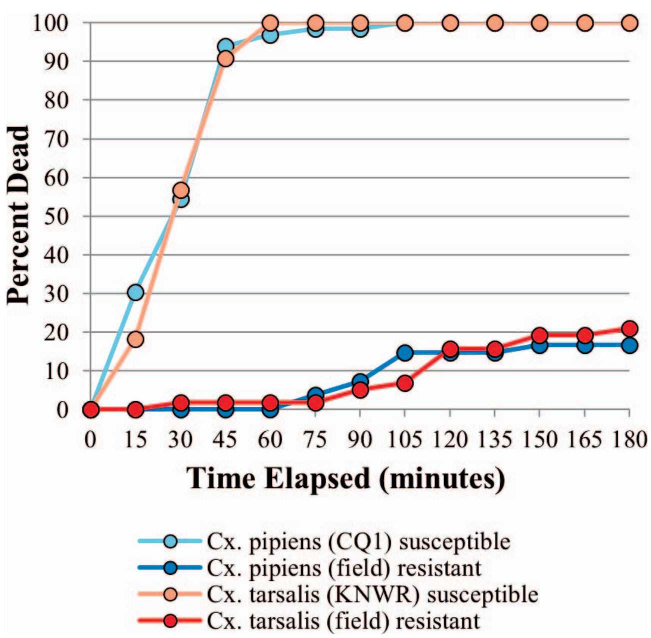

Figure 2.—Time mortality curves showing results of a bottle bioassay performed at the SYMVCD during the 2019 season. Two colony reared susceptible, and two resistant field-collected adult mosquito species were tested using a permethrin chemical standard.

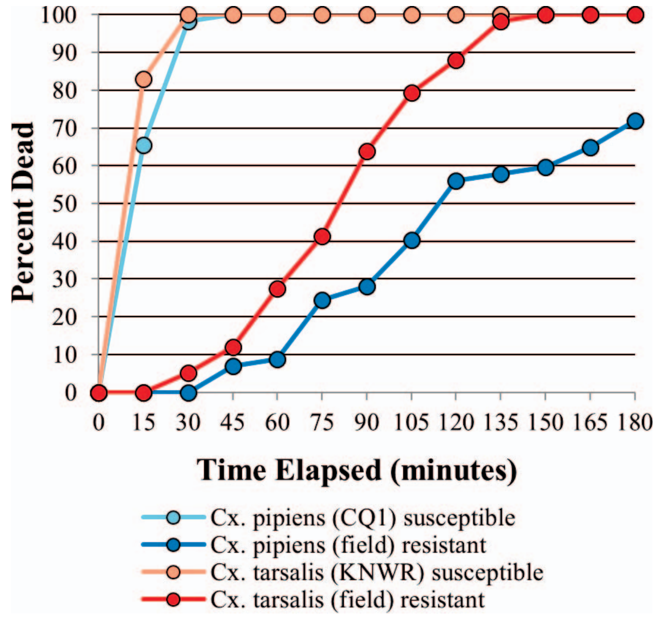

Figure 3.—Time mortality curves showing results of a bottle bioassay performed at the SYMVCD using a permethrin chemical standard combined with a piperonyl butoxide (PBO) chemical standard. These were tested against two colony reared susceptible and two resistant field-collected adult mosquito species during the 2019 season.

Field evaluation of DeltAGard resulted in 100% mortality in nearly all mosquitoes regardless of species or susceptibility (Fig. 4). These results were compared to bottle bioassays using a deltamethrin standard against both *Cx. pipiens* and *Cx. tarsalis*. Each of the bottle

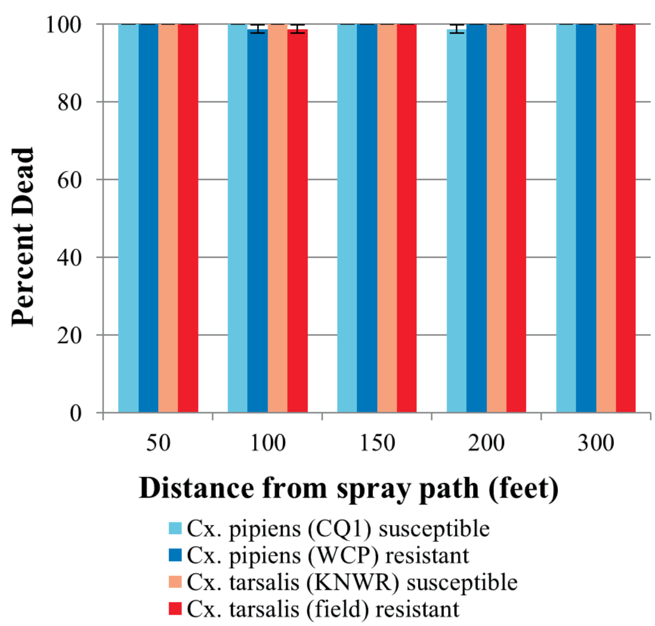

Figure 4.—Mortality at 12 h post application of four groups of adult mosquitoes exposed in sentinel cages during a 2018 field evaluation of the adulticide DeltAGard in Sacramento County, CA.

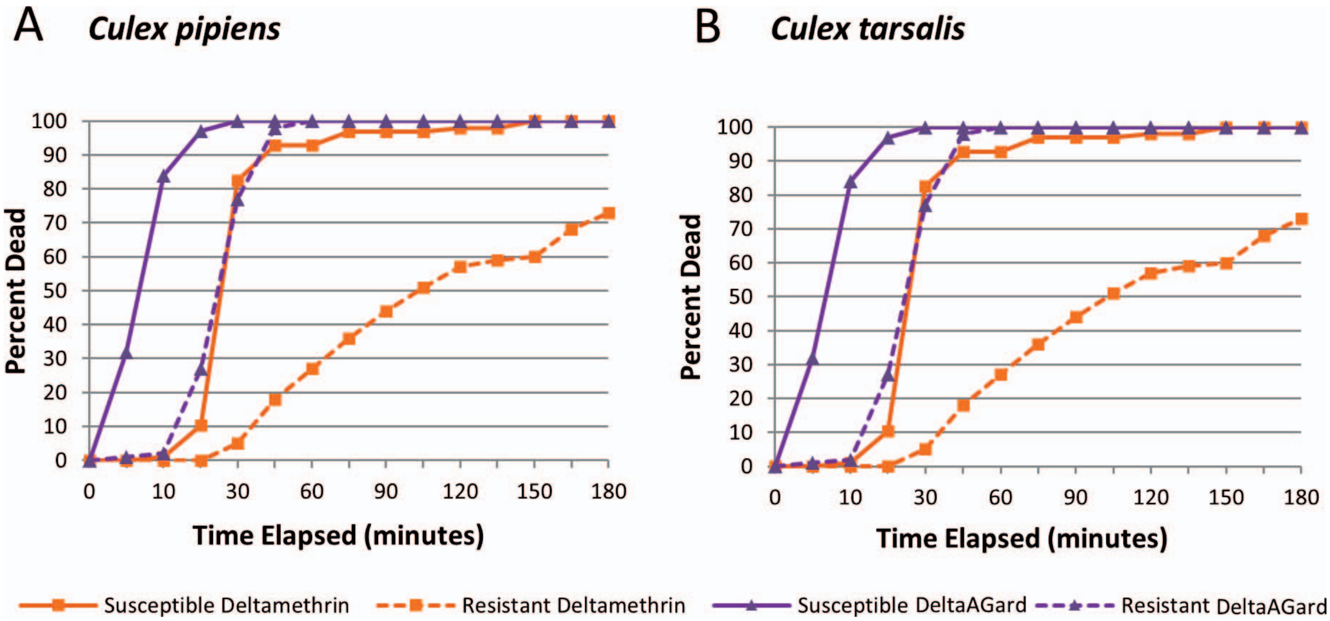

Figure 5.—Results of two separate bottle bioassays performed at SYMVCD designed to evaluate the presence of resistance in adult mosquitoes to the active ingredient deltamethrin. Chemical standard deltamethrin was compared to the formulated product DeltAGard. Test mosquito species represented in graph A are susceptible, colony reared CQ1 strain of *Cx. pipiens* and resistant colony reared Woodland *Cx. pipiens*. Test mosquito species represented in graph B are susceptible, colony reared Kern National Wildlife Refuge strain of *Cx. tarsalis* and resistant colony reared Conaway *Cx. tarsalis*.

bioassays utilized a colony reared susceptible, a colony reared resistant, and 4 wild collected populations of each species. Mortality was 100% for the susceptible populations of both species after 75 minutes of exposure in the bottle. Percent mortality for both colonized resistant and field collected populations of *Cx. pipiens* were $\leq$70% after 75 minutes. Results for *Cx. tarsalis* colonized resistant and field-collected showed 78% mortality after 75 minutes. The Centers for Disease Control bottle bioassay guidelines state that mortality below 90% implies resistance and recommends use of alternative materials and methods for control of mosquito populations. However, the outstanding field performance of this new pyrethroid based product brought this conclusion and course of action into question. Formulated products contain "other ingredients" (as stated on the label) in addition to the active ingredient. These ingredients may improve the efficacy of the product and therefore invalidate the results from using chemical standards alone for assessing the susceptibility of mosquito populations to a formulated product. To better understand how formulated products compared to chemical standards, bottle bioassays were conducted using DeltAGard and a deltamethrin chemical standard. Both products were tested against susceptible and wild colonized populations of *Cx. pipiens* and *Cx. tarsalis* (Fig. 5). There was a substantial offset in the time-mortality curve between the chemical deltamethrin standard and the formulated DeltAGard product tested against the same mosquito populations.

Conclusion

Commercial products commonly contain other ingredients to increase product efficacy and reduce application rate. However, recently developed products may contain undisclosed ingredients instead of traditional synergists such as PBO. Our study revealed the possibility of misleading resistance profile data on mosquito populations of interest when traditional resistance testing approaches utilized chemical standards alone. We did find that bottle bioassay results more accurately aligned with results of operational applications when the synergist in the commercial product formulation was also included in the bottle bioassay with the main active pesticide standard. In cases where the other ingredients are proprietary, our data suggest that use of the formulated product in bottle bioassays is superior to use of only the active ingredients in evaluating mosquito susceptibility. Data presented by Katherine Brisco in the 2019 bottle bioassay training workshop showed a correlation between 2-hour results in the bottle and 12-hour post ULV cage results. Future studies comparing field performance against bottle bioassay results will be performed on other emerging adulticides to see if the link is universally applicable.

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Development of a *Culex kdr* Assay for the Detection of Pyrethroid Resistance

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Introduction

Many species of mosquitoes within the *Culex* genus are vectors for pathogens such as West Nile virus (WNV), St. Louis Encephalitis (SLEV) and filariasis (Farajollahi et al. 2011). Chemical controls, among other measures, are used to mitigate the spread of vector borne diseases, but may result in pesticide resistance. A single nucleotide polymorphism (SNP) in the knockdown resistance (*kdr*) locus of the *voltage gated sodium channel* (*vgsc*) gene of *Culex* mosquitoes confers knockdown resistance to pyrethroids. The most common mutation conferring pyrethroid resistance among *Culex* species is the L1014F mutation. PCR-based assays that detect these SNPs in *Culex* species are currently available only for *Culex pipiens* and *Culex quinquefasciatus* (Chen et al. 2010). Under the threat of widespread resistance, we sought to develop a quantitative reverse transcriptase (qRT)-PCR assay that detects the most common *kdr* mutation in *Culex* species that leads to pyrethroid resistance. Our original goal was to develop this assay for use in *Culex tarsalis*. However, after comparing the cDNA sequences of other *Culex* vectors, we discovered that the qRT-PCR method created a more conserved template compared to its quantitative PCR counterparts, allowing the assay to perform for multiple *Culex* species.

Methods

We designed primer and probe sequences (Integrated DNA Technologies, Coralville, Iowa) using Primer3Plus software based on the *Cx. tarsalis vgsc* complementary DNA sequences (Table 1). Mosquitoes were collected from various trap sites within Alameda County and their RNA was isolated using the MagMAX – 96 Viral RNA Isolation Kit (ThermoFisher Scientific) and the *kdr* single nucleotide polymorphism evaluated using qRT-PCR. Briefly, each RT-PCR reaction featured a volume of 25 microliters consisting of 6.25 microliters Taqman™ Fast Virus 1-Step Master Mix (Thermo Fisher Scientific, Waltham, MA), 2.25 microliters (0.9mM) of RT*kdr*_Fwd and RT*kdr*_Rev primers, 0.6 microliters (0.25mM) of RT*kdr*_WT and RT*kdr*_Mutant probes, 1 microliter of RNA template and 12.05 microliters of nuclease free water. Cycling conditions were based on TaqMan Fast Virus 1-Step Master Mix's Fast Cycling Mode and are as follows: 50°C for 5 minutes, 95°C for 20 seconds, followed by 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds. The *Culex* RT-PCR *kdr* assay was validated through sanger sequencing of the PCR products.

Results and Discussion

We ascertained that a substantial increase in FAM or HEX fluorescence indicated homozygous wildtype or mutant genotype, respectively. An increase in both FAM and HEX in relatively equal fluorescence indicated a heterozygous genotype (Fig. 1). Using sequencing as a reference, we determined the accuracy of the *Culex* RT*kdr* assay to be 99% (not shown). We tested 1,383 *Culex* specimens collected from Alameda County with the *Culex* RT*kdr* assay and found 362 (26%) were resistant, 285 (21%) were heterozygous, and 736 (53%) were susceptible. The resistant allele frequency was 0.57 among *Cx. pipiens*, 0.15 among *Cx. tarsalis* and 0.00 among *Culex erythrothorax*. *Culex pipiens* complex mosquitoes are notorious for their resistance. Prior studies also found high resistant allelic frequencies among *Cx. pipiens* mosquitoes (Yoshimizu et al. 2020, McAbee et al. 2003, Ahmed et al. 2012). Additional, we discovered that *Cx. pipiens* and *Cx. tarsalis*

Table 1.—Primer and probe sequences used in the *Culex* RT*kdr* assay.

Name	Sequence (5′ -> 3′)
Primers	
RTSeq_Fwd	ATCTGACGTTTGTGCTCTGC
RT*kdr*_Fwd	CCTGCATTCCGTTCTTCTTG
RT*kdr*_Rev	GCGATCTTGTTCGTTTCGTT
Probes	
RT*kdr*_WT	FAM-GGTTAAGTA/ZEN/ CGACTAAGTTTCCTATCACTAC-3IABkFQ
RT*kdr*_Mutant	HEX-GGTTAAGTA/ZEN/ CGACAAAGTTTCCTATCACTAC-3IABkFQ

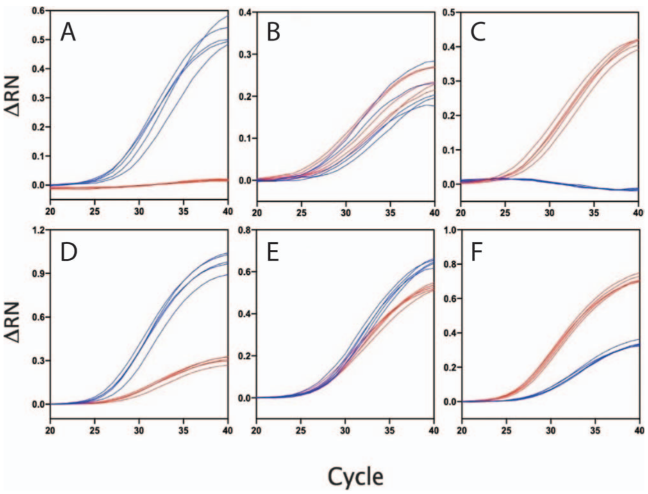

Figure 1.—Amplification plots (ΔRN vs Cycle Number) for *Culex pipiens* (A-C) and *Culex tarsalis* (D-F) with 5 representative specimens selected for each genotype. The blue and red lines represent amplification of the wildtype and mutant probes, respectively. (A) *Culex pipiens* homozygous wildtype; (B) *Culex pipiens* heterozygous; (C) *Culex pipiens* homozygous mutant; (D) *Culex tarsalis* homozygous wildtype; (E) *Culex tarsalis* heterozygous; (F) *Culex tarsalis* homozygous mutant.

mosquitoes from the inland region of Alameda County were more resistant than their coastal counterparts, with resistant allelic frequencies of 0.54 and 0.21, respectively (Fig. 2). The *Cx. erythrothorax* mosquitoes were collected from constructed marsh habitats within the coastal region where they may be exposed to all manner of surface runoff that contain pyrethroid insecticides. That none of the *Cx. erythrothorax* contained an allele that is associated with pyrethroid resistance suggests that if the runoff contained pyrethroids, it had been degraded or diluted to the point of being functionally inactive. According to the California Pesticide Information Portal (Calpip) database, pyrethroids applied in the County are mainly for agriculture and commercial pest control. Runoff due to rainfall is likely transporting pyrethroid residues to mosquito larval habitats, possibly contributing to resistance in the inland region of the County (Tang et al. 2018).

Conclusions

Despite Alameda County Mosquito Abatement District applying less than 10 ounces of adulticides between 2010 and 2019, pyrethroid resistance remains prevalent in Alameda County. Commercial use of pesticides for both structural and agricultural control of pests may be contributing to the higher proportion of resistance we observed inland. Additionally, species behavior and habitat preferences may contribute to insecticide resistance. The *Culex* RT*kdr* assay not only satiates the need for a *Cx.*

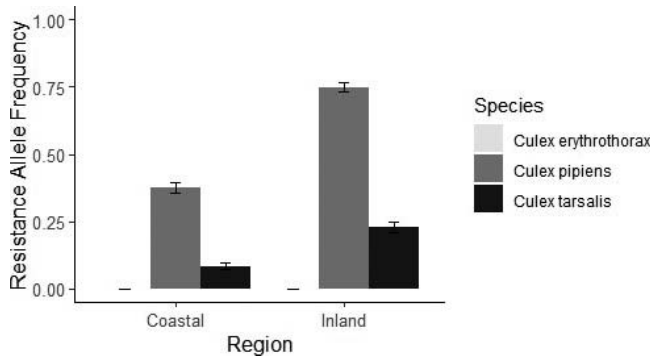

Figure 2.—Resistant allele frequency (F_R) by species and region. Light Grey, dark grey and black bars represent F_R for *Culex erythrothorax* (no resistance detected), *Culex pipiens* and *Culex tarsalis*, respectively.

tarsalis PCR pyrethroid detection assay, but also allows for testing multiple *Culex* species on one PCR run.

Acknowledgements

Dr. Pete Dailey for his unwavering support and guidance as I pursued my master's degree at UC Berkeley and Chris Hoover for his creation of pristine figures in R.

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Summary of biochemical and target site resistance pathways detected in California *Aedes aegypti*, 2017-2018

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Abstract

Aedes aegypti mosquitoes collected in 2017-2018 by local vector control agencies in the Central Valley and southern California were tested by the California Department of Public Health, Vector-Borne Disease Section using biochemical assays to quantify indicators of potential pyrethroid and organophosphate resistance. Assays measured detoxification enzyme expression levels (α-esterases, β-esterases, and mixed-function oxidases) and a propoxur (carbamate) based assay measured acetylcholinesterase target site insensitivity of wild-caught mosquitoes compared to a known susceptible laboratory strain. Results revealed that wild-caught mosquitoes from throughout the sampled areas had elevated levels of mixed-function oxidases and (in)sensitive acetylcholinesterase, whereas α- esterase and β-esterase levels varied among sampled locations. Findings suggest that recently introduced populations of *Ae. aegypti* in California may have functional resistance pathways that could reduce the efficacy of currently available mosquito adulticides.
For more information on this study, see
Yang, F., S. Schildhauer, S. A. Billeter, M. H. Yoshimizu, R. Payne, M. J. Pakingan, M. E. Metzger, K. A. Liebman, R. Hu, V. Kramer, and et al. 2020. Insecticide resistance status of *Aedes aegypti* (Diptera: Culicidae) in California by biochemical assays. J. Med. Entomol. Epub ahead of print.

Efficacy and Longevity of the Insect Growth Regulator Sumilarv® 0.5G (Pyriproxyfen) against *Culex quinquefasciatus* under Semi-Natural Conditions in Orange County, California

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Abstract

Artificial containers found in residential properties play an important role in mosquito production and arbovirus transmission in urban/suburban landscapes and are difficult to control on a regional scale. An ideal mosquito larvicide is one that provides effective, long-term control and limits retreatments to peridomestic sources. From May – October, 2019, Sumilarv® 0.5G, a sand granule formulation with the insect growth regulator pyriproxyfen (0.5%) as active ingredient (a.i.), was evaluated in mesocosm trials using ten 125 liter (L) (33 gal) and four 1820 L (480 gal) tanks to determine the product's effective duration against immature *Culex quinquefasciatus* Say. Tanks were lined with plastic sheeting to prevent pyriproxyfen absorption by the tanks. Eight 125 L tanks were treated with a single 10 g application of Sumilarv 0.5G granules. Three 1820 L tanks were treated with single 10 and 30 g applications of the product in separate trials after changing the plastic lining and water. Two 125 L tanks and one 1820 L tank were used as untreated controls. Wild or laboratory colony *Cx. quinquefasciatus* larvae or egg rafts were added approximately every 14 days to the 125 L tanks (wild or lab colony) and 1820 L tanks (wild only). Pupal samples were transferred to rearing chambers and evaluated for emergence inhibition (EI). Trials ended when $<$ 70% EI was observed in all treated tanks. In total, ten tests were conducted with the 125 L tanks (10 g application), and five (10 g application) and four (30 g application) tests, respectively, were completed with the 1820 L tanks. No adult emergence was detected until 125 days post-treatment in the 125 L tanks (nominal dose 0.40 ppm a.i.) and until 36 and 25 days post-treatment in the 1820 L tanks (nominal doses 0.0824 ppm and 0.0274 a.i., respectively). Emergence inhibition fell below 70% at 48 days post-treatment during the 10 and 30 g trials in the 1820 L tanks. Sumilarv 0.5G demonstrated effective, long-term control in small volume water features; however, given the variability in treatment applications on the label, effective control of immature *Cx. quinquefasciatus* in large water volumes for more than 30 days may not be realized.

INTRODUCTION

Residential water-holding containers are important mosquito sources and are often difficult for mosquito control agencies to monitor and abate. In 2015, the Orange County Mosquito and Vector Control District (OCMVCD) found that 6-7% of the county's residential properties had containers with mosquito breeding, which equates to thousands of small isolated sources (Krueger et al. 2015). Typical examples of artificial peridomestic mosquito sources include neglected pools and spas, stagnant fountains/bird baths, and accumulations of unwanted items or refuse (Ibrahim et al. 2011). Residual-acting larvicidal products are important tools to control residential mosquito breeding when containers cannot be readily eliminated, emptied, or when a resident is unlikely to address the issue in a timely manner (Darriet and Corbel 2006). A larvicide that can provide effective residential mosquito control beyond a typical duration of 14 to 30 days in water features of varying sizes and material compositions would minimize re-applications and are of great interest to mosquito control programs. Larvicidal products formulated with the insect growth regulator (IGR) pyriproxyfen (Sumitomo Chemical Co., Ltd., Tokyo, Japan) may offer relatively long-term mosquito suppression. However, duration and efficacy of control may be affected by the chemical composition of the water-holding containers, among many other factors.

Pyriproxyfen is an IGR in the Insecticide Resistance Action Committee mode of action group 7C, a juvenile hormone mimic, and works to prevent emergence of healthy adults from pupae (WHO 2001). At high concentrations, it can cause mortality in recently deposited and fully embryonated eggs and inhibit pupation, resulting in larval death (Ishaaya and Horowitz 1992, Kamal and Khater 2010, Mbare et al. 2013, Suman et al. 2015).

Pyriproxyfen has been used for larval mosquito control in many countries since the 1990s (Sihuincha et al. 2005, Invest and Lucas 2008) and was registered in the USA for agricultural use in 1996 to control white flies (Devillers and James 2013). In addition, it has been used widely to control animal ectoparasites, bed bugs, and fire ants (Sullivan 2000). Pyriproxyfen has been commercially-available in the liquid product NyGuard® (MGK, MN, USA) since 2003 in California (CDPR 2020) for control of a wide variety of flying and crawling arthropods of urban, residential, and public health importance, including mosquitoes (Su et al. 2019).

The mosquito larvicide Sumilarv® 0.5G (pyriproxyfen 0.5%, EPA No. 1021-2819, MGK) was registered in the US and California in 2010 and 2019, respectively. The product is formulated with a fine sand substrate for slow release of the active ingredient (a.i.) pyriproxyfen and can be applied directly to isolated water sources not connected to waters of the US. It has shown effective residual mosquito control beyond 30 days in several studies (Vythilingam et al. 2005, Invest and Lucas 2008, Elkashef et al. 2019).

Pyriproxyfen may bind to, or be sequestered by, various substrates (MGK 2020), which may impact its efficacy and control duration. When applied to isolated concrete catch basins for immature mosquito control in Sacramento, CA, a single 70 g dose of Sumilarv 0.5G showed efficacy for approximately 150 days (Elkashef et al. 2019). The authors suggested that pyriproxyfen was sequestered in the concrete, and released later, allowing for extended mosquito control, even after water had been completely depleted and refilled in the stormwater basins.

Our study was not designed to test various substrate materials but to better understand the efficacy and the duration of control of *Culex quinquefasciatus* Say larvae after a single application with Sumilarv 0.5G to artificial plastic containers, which are ubiquitous among residential properties in Orange County (Krueger et al.2015). *Culex quinquefasciatus* is the dominant mosquito found in peridomestic sources in Orange County (Schreiber et al. 1989) and is the primary urban vector of West Nile virus in southern California (Kwan et al. 2010). Pyriproxyfen is effective against *Cx. quinquefasciatus* larvae and is reported to be stable in the field as a granular formulation (Mulla et al. 1986). It also has minimal environmental impacts at recommend application rates for mosquitoes (Schaefer et al. 1988).

EXPERIMENTAL DESIGN AND METHODS

The residual activity of Sumilarv 0.5G sand granules on *Cx. quinquefasciatus* larvae was evaluated for 140 days from May 28 – October 15, 2019, in two mesocosm configurations consisting of either ten "medium" (35.81 cm x 48.26 cm x 89.66 cm) or four "large" (130 cm x 250 cm x 90 cm) tanks lined on the inside with 0.102 mm (0.004 in.) low-density polyethylene plastic (LDPE) sheeting. Each mesocosm configuration held 125 liters (L) (33 gal) or 1820 L (480 gal), respectively. The plastic sheeting was used to prevent absorption of pyriproxyfen by the tanks and mimic a common type of plastic (LDPE) comprising many

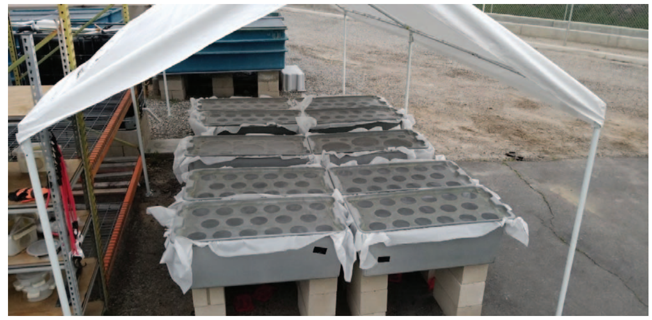

Figure 1.—Arrangement of ten medium-sized tanks (eight treatment tanks and two controls) for testing the efficacy of Sumilarv 0.5G against field and laboratory colony *Cx. quinquefasciatus* larvae.

container sources on residential properties. Water temperatures were monitored with high/low mercury thermometers submerged in the tanks. No measurable rainfall was recorded during the study.

One day prior to each test, adult mosquitoes were collected at the OCMVCD headquarters (Garden Grove, CA) in gravid traps (Cummings 1992). After processing, gravid *Cx. quinquefasciatus* were transferred to holding cages for egg laying in oviposition media. Egg rafts from OCMVCD's *Cx. quinquefasciatus* laboratory colony (pyrethroid-resistant strain collected from Chino Hills and Montclair, CA, in 2006, Tianyun Su, personal communication) were also collected at the same time. Throughout the experiments, wild and laboratory egg rafts were removed from their respective cages and introduced into designated treatment and control tanks. All experiments were conducted using larvicide from a single package of Sumilarv 0.5G (LOT 19101F4).

Medium (125 L) Tank Experiment

Ten plastic-lined, medium-sized tanks (polystyrene resin fiberglass) were fitted with lids modified with equally-spaced, insect-screened, circular holes comprising approximately 37% of the lid's surface area for venting and natural light exposure (Fig. 1). Each tank was filled with approximately 125 L of tap water to a depth of 26.67 cm at the beginning of the experiment. A single square swatch of plastic sheeting (30.5 cm x 30.5 cm) was submerged into each tank of water. Each time pupal samples were removed from a tank for rearing, the swatch was also removed and used to line a mosquito emergence chamber and returned to the tank at the end of each emergence observation period. A canopy was used to shade the tanks from direct sunlight and limit evaporation. Water was added as needed to maintain a minimum depth of 10 cm. The tanks held either wild or laboratory colony *Cx. quinquefasciatus* larvae. Eight tanks were used for treatment and two tanks were used as controls, with four treatment tanks and one control for each wild or colony strain.

On May 28, 2019, 300-400 second instar *Cx. quinquefasciatus* larvae (wild or lab colony) were introduced to each designated tank, and after an hour of acclimation,

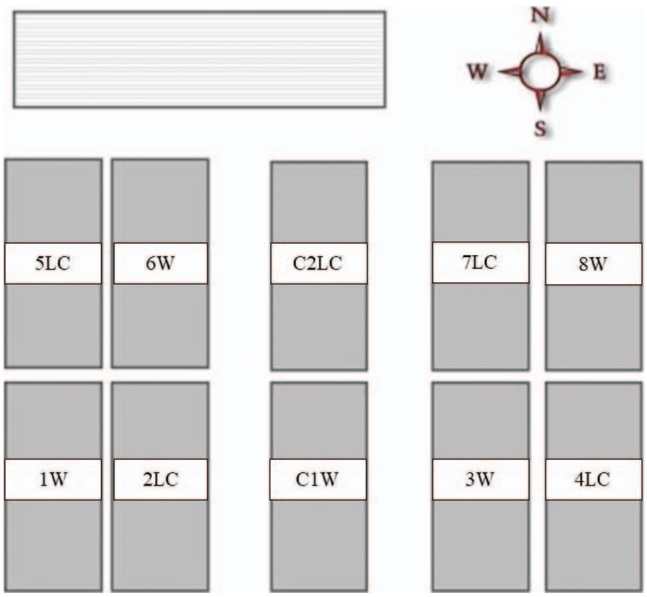

Figure 2.—Schematic of medium-sized tank block design. Tanks with wild (W) and laboratory colony (LC) *Cx. quinquefasciatus* larvae were alternated across rows with control tanks in the middle of the block.

10 g of Sumilarv 0.5G (nominal dose 0.40 ppm a.i.) were sprinkled evenly over the water surface by hand into each of eight treatment tanks per label specifications [10 g/0-1892 L (0-500 gal), $\leq$ 30.5 cm (1 ft.) depth] (Fig. 2). All ten tanks were supplied individually with approximately 20 g (0.16 g/L) of rabbit feed (Complete Feed for Rabbit, Mana Pro, MO), and more pellets were added as needed to promote microorganism growth. Thereafter, 5-10 egg rafts (wild or lab colony) were introduced to each designated tank at approximately 14 day intervals, allowing larvae to hatch directly into the water. If pupae developed, 50 pupae (or less) were transferred with 371 ml of tank water to a specified 0.95 L emergence chamber (#1425, BioQuip Products, Inc., Rancho Dominguez, CA) lined with the plastic swatch taken from the respective tanks (treatment and controls) (Fig. 3). Emergence chambers were held indoors on a 12/12 hr. light-dark cycle at 25°C. Emergence was monitored daily in the rearing chambers until 100% adult emergence or pupal death (72-96 hrs.).

The plastic liner and treated water were used in the rearing chambers to continue to expose transferred pupae from the treatment tanks to pyriproxyfen. Once a test was finished, the plastic sheeting and emergence chamber water were returned to the corresponding tank to best conserve the pyriproxyfen concentration in the treatment tank. This sampling procedure was repeated until emergence inhibition (EI) fell below 70% in the treated tanks. In total, one batch of larvae and nine batches of egg rafts were introduced into the tanks, from which nine cohorts of 50 pupae (or less) were sampled and observed. Tank water temperatures ranged from 14.4-28.3° C.

Figure 3.—Emergence chamber lined with plastic sheeting.

Large (1820 L) Tank Experiment

Four large, plastic-lined tanks were fitted with removable, wood-framed screened coverings, and filled with 1820 L of water to a depth of 58.4 cm (Fig. 4). Unlike the 125 L tank experiment, the water depth was maintained during each test by topping off the tanks on alternate days with small amounts of water to compensate for evapora-

Figure 4.—Arrangement of the large tanks (three treatment tanks and one untreated control tank, foreground) with wood-framed, screened covers.

Figure 5.—Floating bioassay cages.

tion. Large tanks held only wild *Cx. quinquefasciatus* larvae, with one tank used as an untreated control. Because the volume and depth (1820 L, 58.4 cm) of the large tanks were between label application rates of 10 g/0-1892 L (0-500 gal) for depths ≤ 30.5 cm (1 ft.) and 30 g/1892-5678 L (500-1500 gal) at depths ≤ 61.0 cm (2 ft.), both application rates were tested. Each tank was supplied with approximately 290 g of rabbit pellets at the beginning of each test, and more pellets were added as needed to promote microorganism growth. Between tests, small mesh hand nets were used to remove any remaining larvae, pupae, and reduce algae (if needed) from the tanks; plastic swatches from the emergence chambers were removed and quickly rinsed with tap water to remove algal growth.

10 gram trial

On May 30, 2019, approximately 1,000 second instar wild *Cx. quinquefasciatus* larvae were placed in each tank, and after an hour of acclimation, 10 g of Sumilarv 0.5G (nominal dose 0.0275 ppm a.i.) were applied evenly over the water surface by hand to each of three treatment tanks. After several days of larval development, 50 free-swimming third and early fourth instar larvae were transferred into a floating bioassay cage (BioQuip Products, Inc., Rancho Dominguez, CA) (Fig. 5) in each tank. The bioassay cages were approximately 21 cm deep x 18 cm diameter, and when floating in the tank, allowed larvae to swim vertically through an 18 cm deep water column. After pupation, 50 free-swimming and 50 caged pupae (if available) were collected, transferred with tank water into designated plastic-lined emergence chambers, and held under controlled laboratory conditions as described earlier. Unlike the medium-sized tank experiment, plastic swatches were not continuously immersed in the test tanks. After the end of each test, swatches were rinsed, air dried, and re-inserted into their respective emergence chambers for re-use. The swatches were used to line the inside of the emergence chambers to match the LDPE plastic lining used in the experimental tanks.

After the initial larval introduction, 5-10 egg rafts were added to each tank, approximately every 14 days until the trial ended. The aforementioned sampling procedures were repeated until mid-August, 2019, when emergence inhibition fell below 70% for two consecutive sampling periods in all treated tanks. In total, one larval batch and four batches of 5-10 egg rafts were introduced to each tank, from which five cohorts of 50 pupae (or less) from free-swimming and caged pupae were evaluated for emergence inhibition. Water temperatures ranged from 19.0-31.8° C. Pupation occurred within 7-12 days after introduction of larvae or egg rafts during the trial. At the end of the 10 g trial, tanks were emptied, plastic linings were replaced, and tanks were refilled with 1820 L.

30 gram trial

On August 30, 2019, wild *Cx. quinquefasciatus* egg rafts (5-10/tank) were introduced to each tank, and 30 g of Sumilarv 0.5G (nominal dose 0.0824 ppm a.i.) were applied evenly over the water surface by hand to each of three treatment tank. Wild *Cx. quinquefasciatus* egg rafts (5-10/tank) were added approximately every 14 days; the same sampling and evaluation methods were repeated as described earlier. In total, four batches of egg rafts, from which four cohorts of 50 pupae (or less) from free-swimming and caged pupae were evaluated for emergence inhibition. Water temperatures ranged from 19.0-32.8° C. Pupation occurred within 7-14 days after introduction of egg rafts during the trial.

Data Analysis

Data from replicates for each treatment were combined for the medium-sized tank experiment and the two large tank experiments separately, and emergence inhibition (EI) was calculated (apps.who.int):

$$EI(\%) = \frac{C - T}{C} \times 100$$

Where T = percentage emergence of treatment, and C = percentage emergence of control. We defined emergence as any adult who separated from the pupal casing and could fly.

An analysis of variance (ANOVA) was used to compare adult emergence between wild and laboratory colony mosquitoes in the medium tanks and between free-

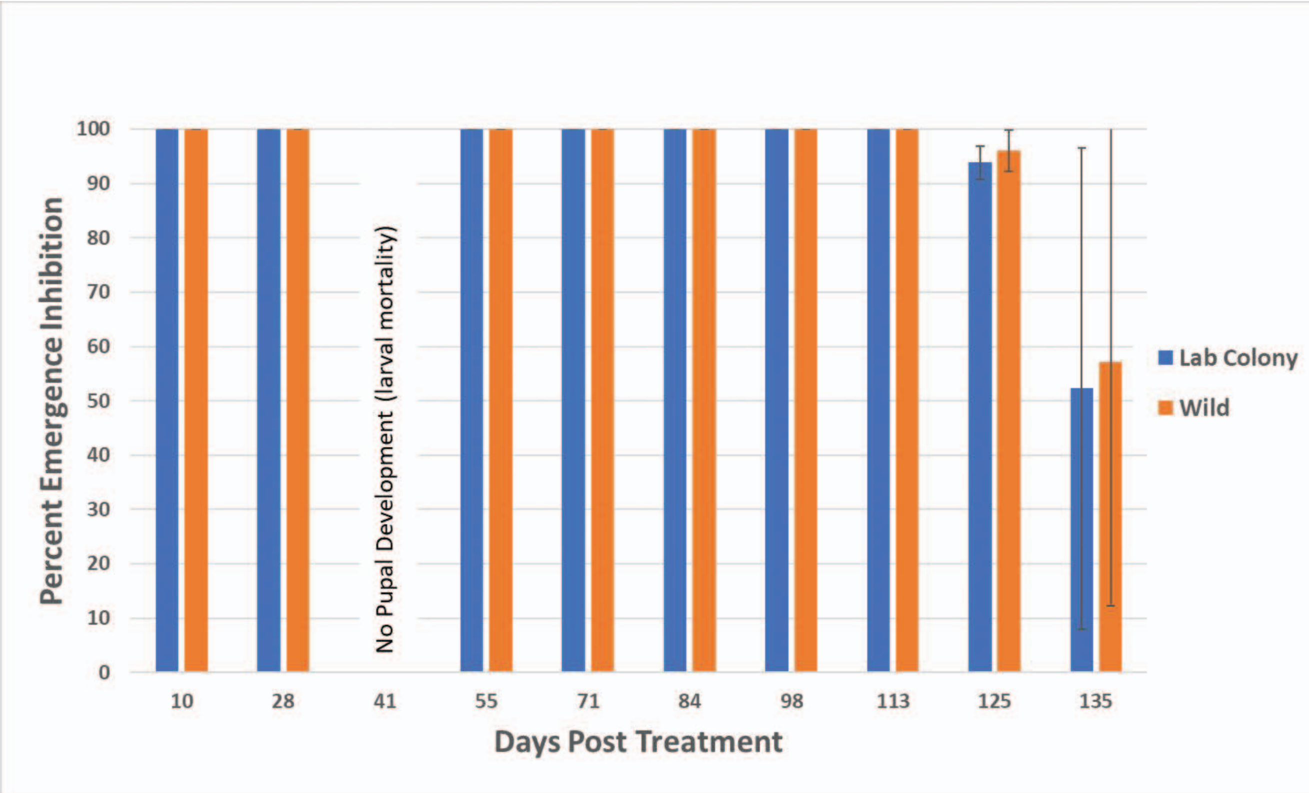

Figure 6.—Emergence inhibition of *Culex quinquefasciatus* after a single 10 g application of Sumilarv 0.5G in medium-sized (125 L) tanks. Mean ± 1 standard deviation (SD).

swimming and caged wild pupae in the large tanks for the 10 and 30 g applications.

RESULTS

Medium (125 L) Tank Experiment: 10 g/tank Sumilarv 0.5G application

The 125 L tank experiment ran from May 28 - October 12, 2019. There was no significant difference in emergence inhibition between wild and colony *Cx. quinquefasciatus* [$F(1,16) = 0.012$, $p = 0.914$] (Figure 6). Apart from no pupal development and extensive larval mortality on day 41, there was 100% emergence inhibition until day 113 post-treatment. On day 125, the emergence inhibition was still approximately 90%. By day 135 post- treatment, < 60% mean emergence inhibition was observed.

Large (1820 L) Tank Experiment: 10 g/tank Sumilarv 0.5G application

Free-swimming and caged pupae exhibited < 70% emergence inhibition by day 25 post-treatment. For both groups, there was a rebound to 80% emergence inhibition on day 36. On day 48, emergence inhibition fell below 70%, and further declined to less than 10% by day 62. Free-swimming larvae in the 1820 L tanks were able to swim and feed throughout the entire water column, whereas caged larvae were confined to the upper 18 cm of the column. There was no significant difference in emergence inhibition between free swimming and caged larvae at the 10 g/tank Sumilarv 0.5G application rate (Figure 7a,b) [$F(1,28) = 0.7258$, $p = 0.4014$].

Large (1820 L) Tank Experiment: 30 g/tank Sumilarv 0.5G application

Free-swimming pupae exhibited 70% emergence inhibition by day 36 post-treatment and fell below 10% by day 48. For caged larvae, emergence inhibition also dropped by day 36 and day 48, but emergence inhibition was still higher compared to free-swimming larvae (82% and 40% respectively). There was no significant difference in emergence inhibition between free-swimming and caged larvae after the 30 g application (Figure 7 c, d) [$F(1,22) = 0.6450$, $p = 0.4304$]. However, there may have been a biologically significant difference between the two groups (free and caged) on day 48 that was not detected statistically due to small sample size. Compared to the rebound effect seen on day 25 in the 10 g/tank trial, there appeared to be no rebound in emergence inhibition in the 30 g/tank trial.

DISCUSSION

The Sumilarv 0.5G label specifies graduated application rates based on water volume and depth for prospective treatment sources. According to the label for water sources 0-1892 L (0-500 gal.) and ≤ 30.5 cm (1 ft.) depth, 10 g is

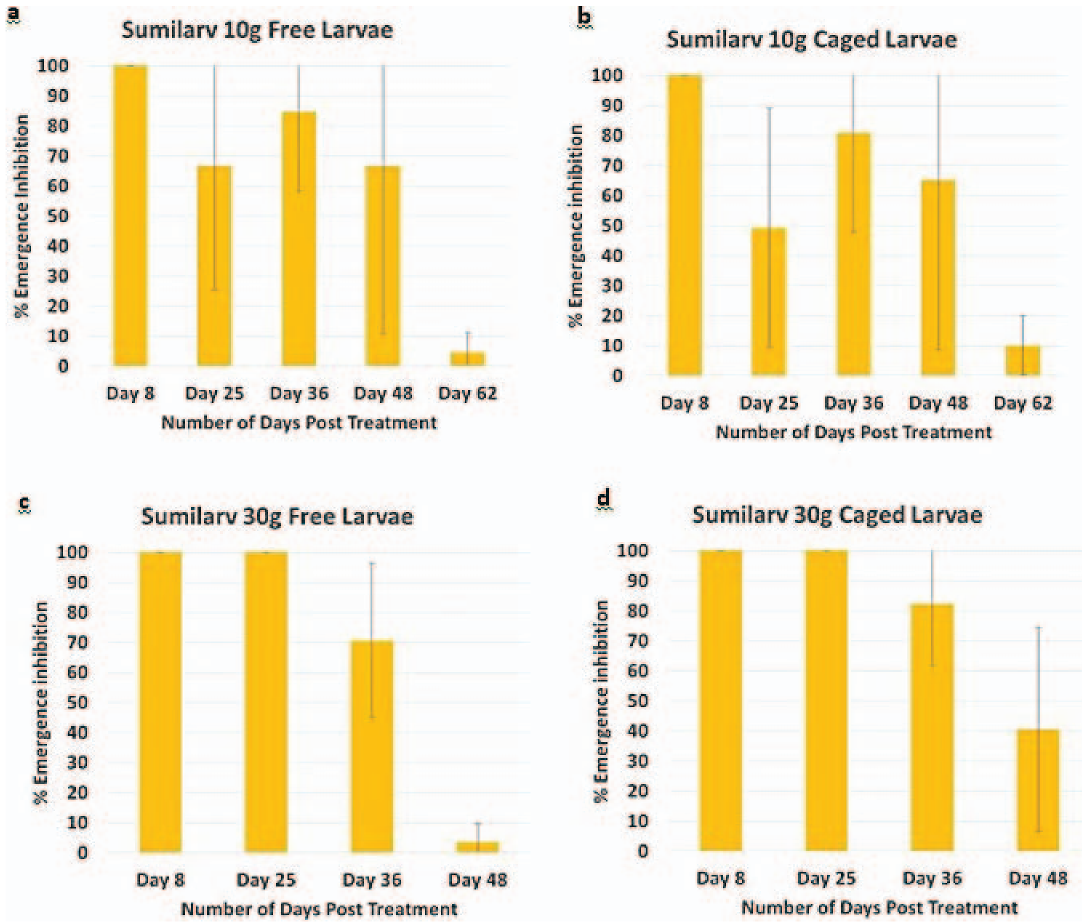

Figure 7.—Emergence inhibition for wild *Culex quinquefasciatus* larvae exposed to a single application of Sumilarv 0.5G in large tanks (1820 L) at applications rates of (a) 10 and (c) 30 g/tank with free-swimming larvae, and (b) 10 and (d) 30 g/tank of caged larvae. Mean $\pm$ 1 standard deviation (SD).

the recommended application; for water bodies 1892 L – 5678 L (500 – 1500 gal) and $\leq$ 61 cm (2 ft.) depth, 30 g is to be applied. This variation in application rates results in a wide range of pyriproxyfen concentrations in treated sources. As described on the label, small volume water sources could receive the same amount of applied product (10 g) within the same application range as larger sources with nearly 1892 L. However, a 10 g application to an 18.2 L bucket and to an 1820 L water source produces two orders of magnitude difference in the nominal pyriproxyfen doses, 2.75 and 0.0275 ppm, respectively. In its present form, the Sumilarv 0.5G label is open for interpretation as to whether volume or depth takes priority when determining the application rate for a treatment site. Water volumes in our large treatment tanks were less than 1892 L but were more than 30.5 cm deep, and so we tested both 10 and 30 g application rates.

A 10 g application of Sumilarv 0.5G in 125 L tanks resulted in a nominal dose of 0.40 ppm pyriproxyfen and produced $>$ 90% emergence inhibition for 125 days. The same 10 g application in the 1820 L tanks resulted in a nominal dose of 0.0274 ppm a.i. (14.7 times lower) and yielded variable results, with emergence inhibition of 100% at day 8, $<$ 70% at day 25, and approximately 80% at day 36. After treatment with 30 g of product, the 1820 L tanks had a nominal pyriproxyfen dose three times higher and sustained 100% emergence inhibition 17 days longer than the 10 g application. At 48 days post-treatment, emergence inhibition decreased for both applications in the 1820 L tanks to less than the 80% level that is recommended by the World Health Organization for successful immature mosquito control (WHO 2005).

To extrapolate from the results of our study, Sumilarv 0.5G could provide acceptable control in small-sized containers for over 90 days, while in large-volume residential water features (neglected swimming pools, ponds, etc.), the specified application rates would likely be efficacious for 30 days or less. Although the concentrations of pyriproxyfen were not measured in this study and estimates of pyriproxyfen levels were calculated based only on the nominal doses of Sumilarv 0.5G, more precise application recommendations should be explored by the manufacture to enable long-term, effective control for all water volumes under the label.

Floating bioassay cages provide a convenient method for sampling pupae in the field or in large test tanks; however, the cage structure may impede water currents and prevent larval movement through areas of high concentrations of active ingredient, such as the water layer near a slow-release product at the bottom of a treated source. We found no statistically significant difference in emergence inhibition between free-swimming larvae and larvae in the floating bioassay cage within the same 1820 L tank for the 10 and 30 g applications. However, there was a marked difference on day 48 between free-swimming and caged larvae when the tanks were treated with 30 g of product, although this difference was not statistically significant. We hypothesize that free swimming larvae incurred exposure to higher concentrations of active ingredient when at the bottom of the tanks, compared to larvae confined in bioassay cages. Measurements of columnar active ingredient concentrations could shed light on the utility of bioassay cages in future studies.

There was extensive larval mortality on day 41 in the 125 L tanks. Although pyriproxyfen concentrations were relatively high in these tanks, the levels were not likely responsible for this larval mortality. Field efficacy trials with granular formulations of 0.5% pyriproxyfen have demonstrated $>$ 90% emergence inhibition in *Cx. quinquefasciatus* but relatively low larval mortality at field application rates (WHO 2001). One possible cause of this unexpected loss of immature mosquitoes was from predation by copepods. Copepods invaded at least three of the tanks and then subsided naturally within two weeks and were not a factor at subsequent pupal sampling points.

As shown in this study, Sumilarv 0.5G demonstrated effective control longer than many of the retreatment intervals seen in other frequently used larvicidal products when applied to shallow, small volume water features. However, given the variability in treatment applications as prescribed by the Sumilarv 0.5G label, effective control of immature *Cx. quinquefasciatus* for more than 30 days in large water volumes with low pyriproxyfen concentrations may not be realized, potentially giving rise to pyriproxyfen resistance in *Cx. quinquefasciatus* from sub-lethal doses. Among commonly used biorational IGR pesticides against mosquitoes, pyriproxyfen is the most active with the lowest lethal concentrations and longest control, which warrants to be preserved by resistance management strategies (Su et al. 2019).

ACKNOWLEDGEMENTS

We thank Jennifer L. Williams and Brian Meagher, MGK, for supplying testing material and study design input. We thank BioQuip for designing the custom-made bioassay larval cages specifically for this experiment. We want to extend our gratitude to Philip Shin and David Taylor, both seasonal assistants from OCMVCD, for their part in the project. Lastly, we would like to recognize the District Manager, Rick Howard, for supporting this important work.

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