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Symposium: Innovation and Discovery in Mosquito Control

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Submitted Papers

William C. Reeves New Investigator Award

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

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

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

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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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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.

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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

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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.

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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.

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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.

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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*.

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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

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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

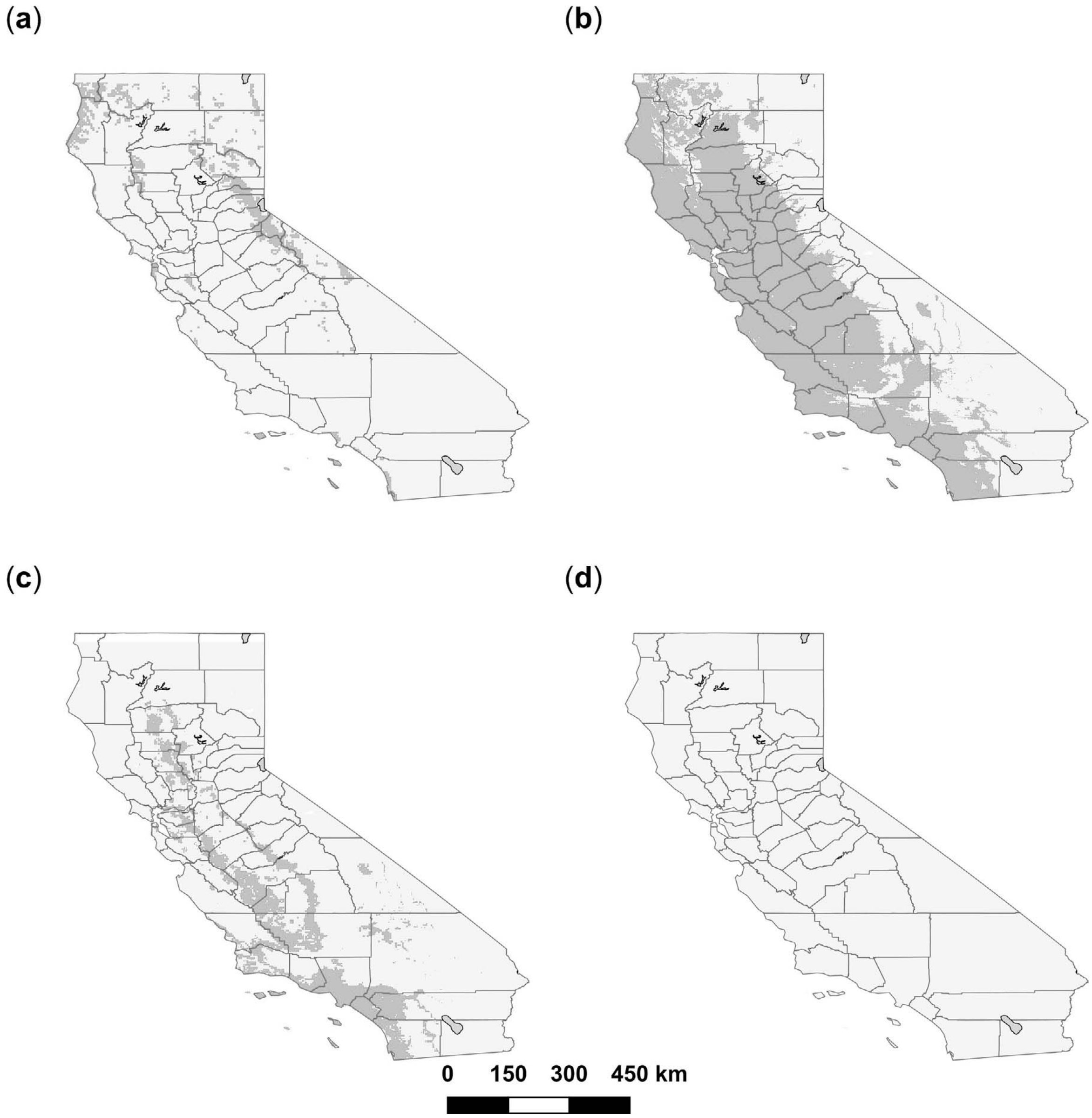

Figure 1.—Habitat in California predicted (using MaxEnt species distribution modeling) to be environmentally suitable for four exotic *Amblyomma* tick species: (a) *A. americanum*; (b) *A. maculatum*; (c) *A. cajennense*; and (d) *A. mixtum*. Areas shaded in dark gray represent potentially suitable habitat 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).

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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.

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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

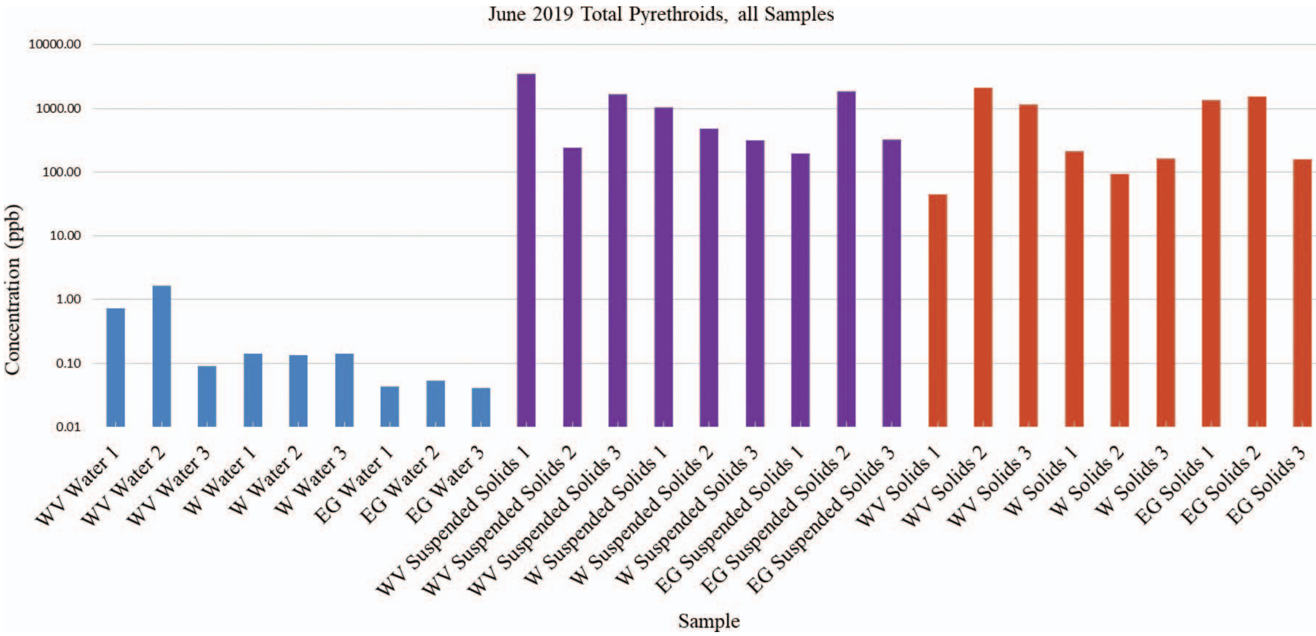

Figure 2.—Pyrethroid concentrations in parts per billion (ppb) in June catch basin samples. Water samples are represented by blue bars, suspended solids by purple, and solids by orange. WV = West Valley (samples from Ontario, CA); W = Woodland; EG = Elk Grove.

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

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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^{TM} 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^{TM} 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).

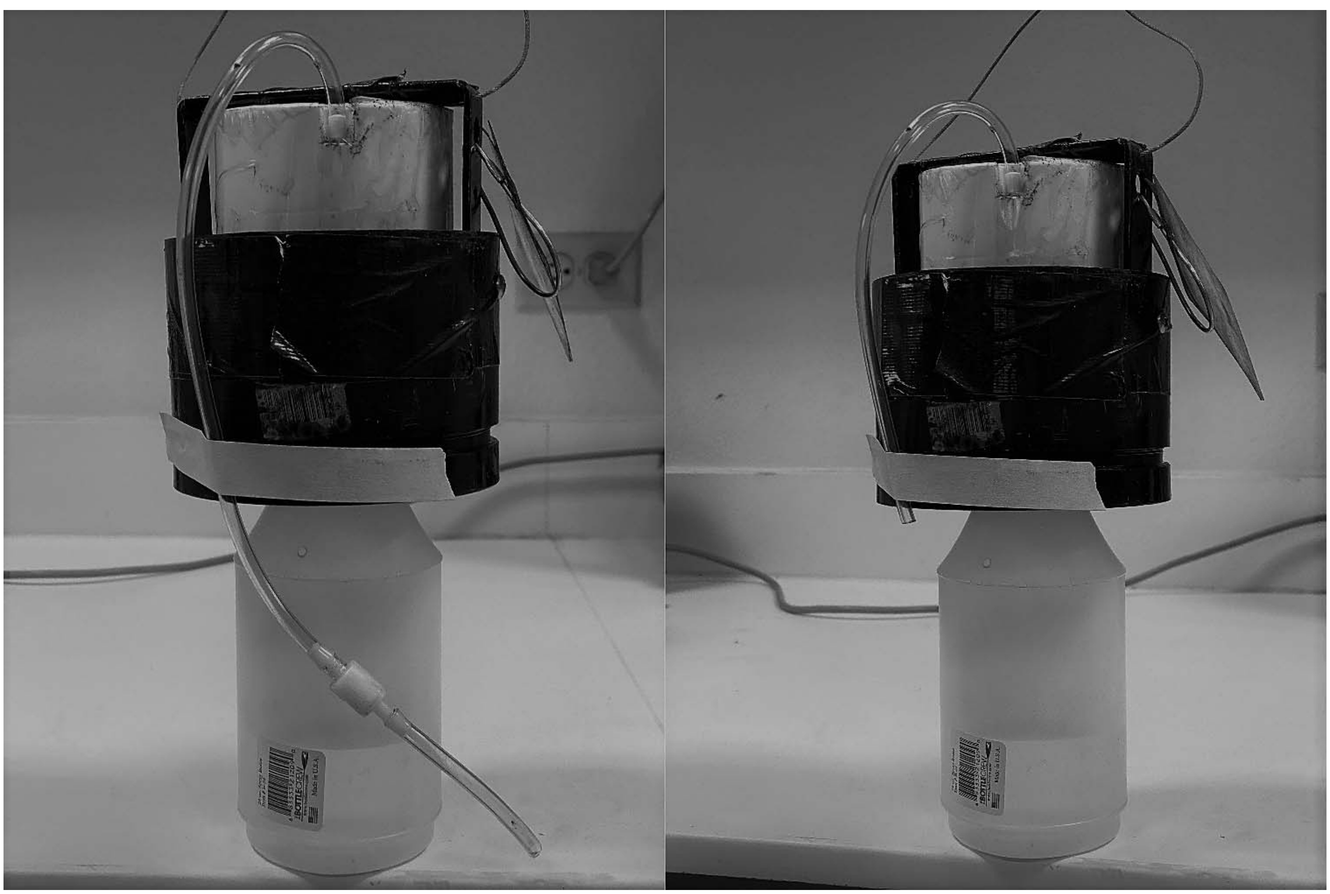

Figure 3.—The left picture shows the outlet tube and check valve with the end of the tube bellow the bottle. The right picture shows a better solution to the siphoning issue; the end of the outlet tube is above the bottle with no need for a check valve.

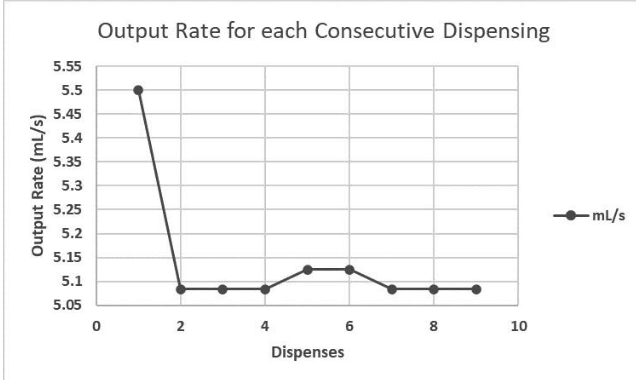

Figure 4.—Change in output rate after consecutive dispensing when a program has been set. The first dispense, after setting the program, has a higher output then the consecutive dispensing.

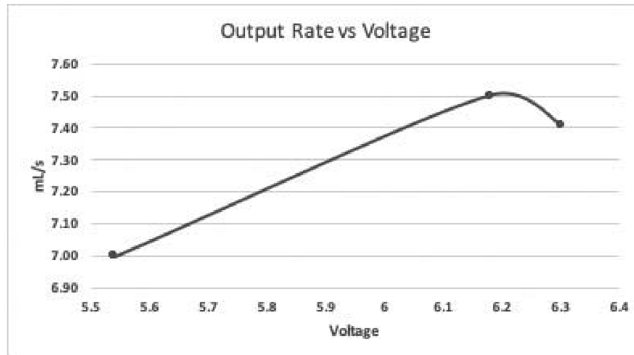

Figure 5.—In manual dispense mode the output rate drops as a function of voltage decrease. Voltage dropped by 0.76 V resulting in a 0.5 mL/s decrease in output.

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™ 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™ 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

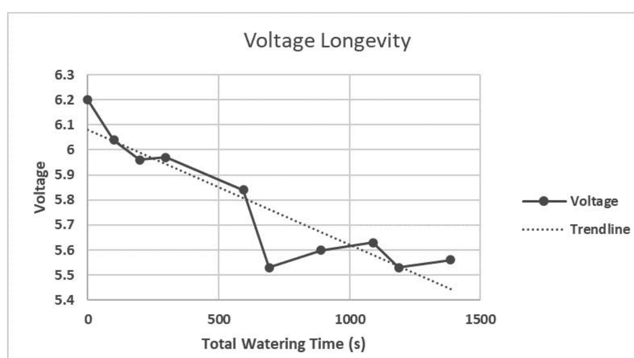

Figure 6.—Test of voltage over time. Voltage dropped immediately after dispensing and then rebounded.

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^{TM} had begun to degrade various plastic parts of the drip system. CocoBear^{TM} 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^{TM} came into contact with moisture it became viscus. This viscus CocoBear^{TM} 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^{TM}. 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^{TM} applications, the District uses a squirt bottle which can be turned down to a mist to cover a much wider area. The CocoBear^{TM} 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^{TM}. Because the system is designed to have a bottle screwed directly to it, it will always come into contact with CocoBear^{TM}. Future systems will require testing with CocoBear^{TM} 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^{TM}, clamps will be needed on the barb fittings to stop them from leaking.

Conclusion

The drip system did dispense CocoBear^{TM} on schedule when there were not any mechanical issues. We feel that this is a proof of concept. CocoBear^{TM} 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

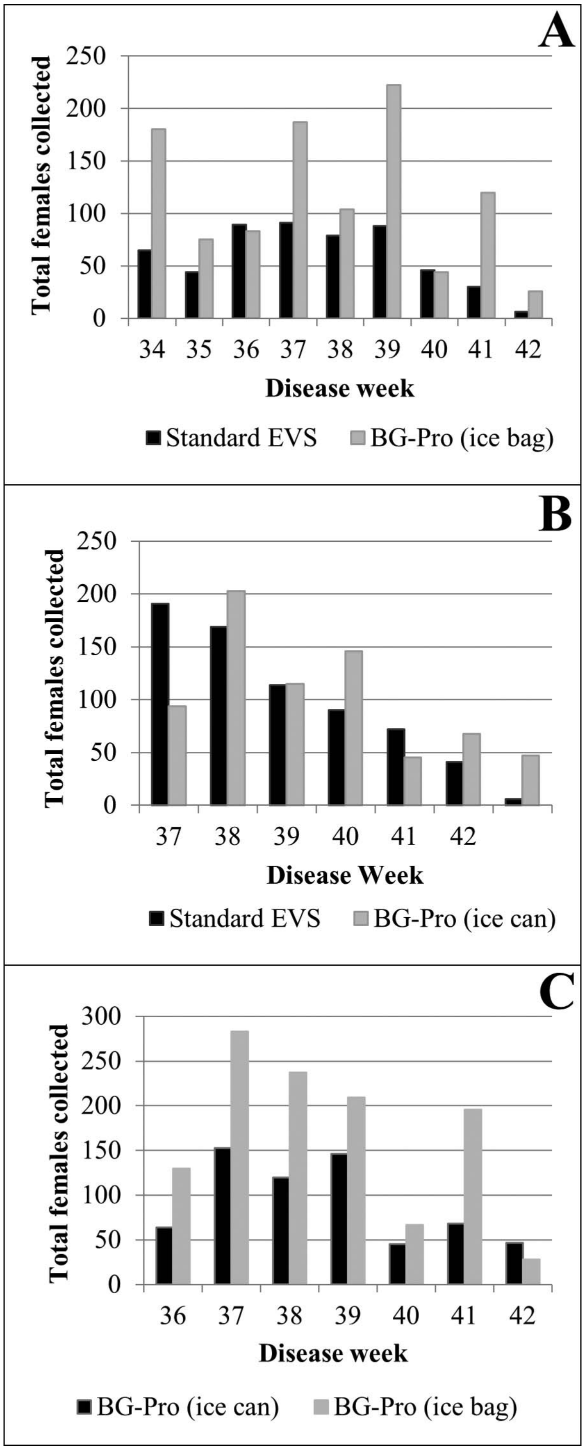

Figure 1.—The total female mosquitoes collected by week in: A. standard EVS traps versus BG-Pro traps set with the manufacture provided ice bag, **B.** a standard EVS trap versus a BG-Pro set with a standard EVS ice can; and **C.** BG-Pro traps set either with the manufacturer provided ice bag or a standard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

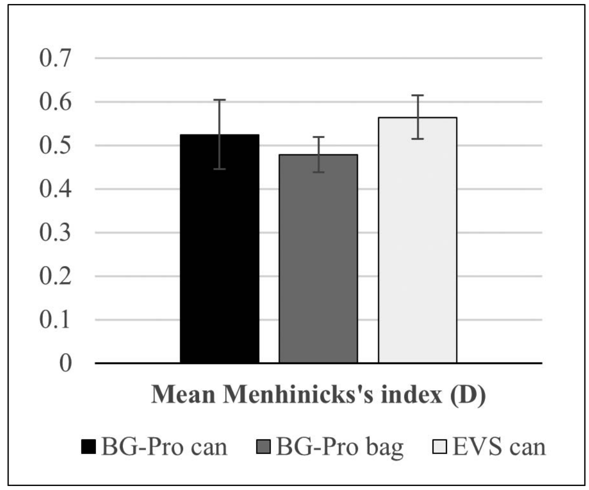

Figure 2.—Overall Species richness by trap type, mean Menhinicks index scores and standard error of the mean are shown.

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

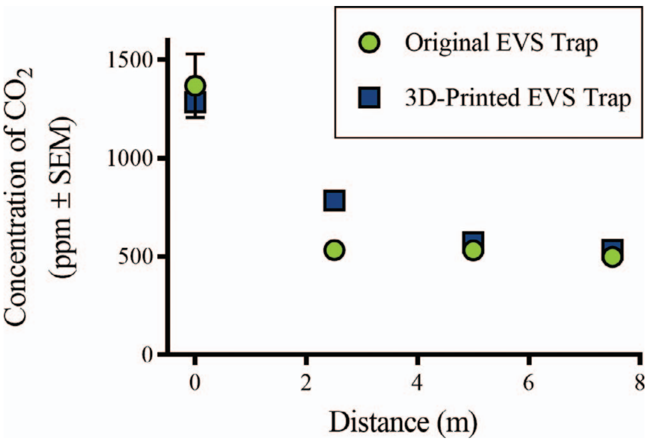

Figure 2.—The dispersal distance of CO_2 from the original EVS trap and the 3D-printed EVS trap.

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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CalSurv Maps. 2020. California Vectorborne Disease Surveillance System. https://maps.calsurv.org. Accessed 1 May 2020.

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

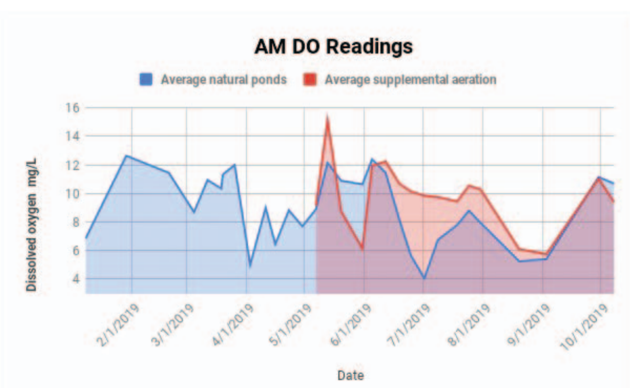

Figure 1.—Dissolved oxygen (DO) readings taken during morning (AM) for aerated and natural (non-aerated) ponds. No major dips in DO were seen in July for aerated ponds. The installed solar aerator prevented DO levels from major fluctuations.

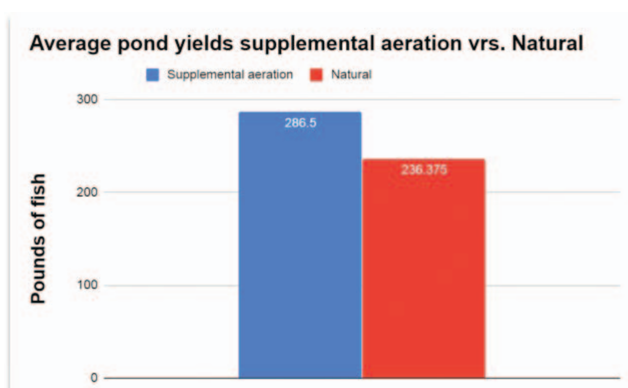

Figure 2.—Overall yields in average pounds of fish in aerated and non-aerated ponds. On average aerated ponds yielded approximately 50 pounds more mosquitofish.

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 **10% heavier**	1.52 **12% heavier**	2.08	1.27
CM	5.2 **4% larger**	2.2 **Same**	5	2.2

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

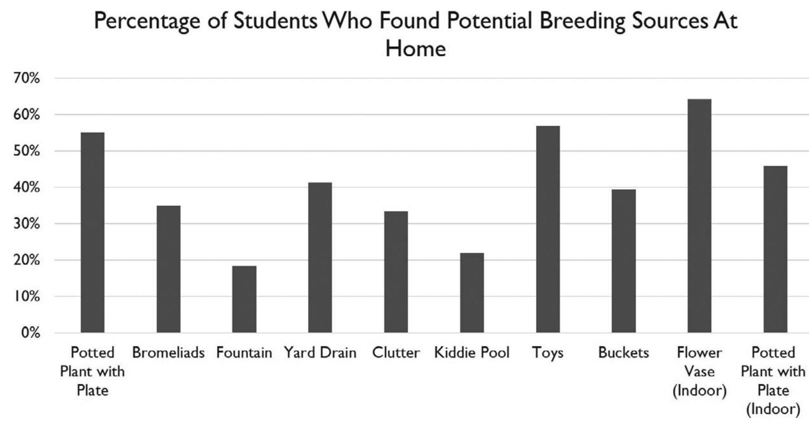

Figure 2.—The percentage of students who found each potential larval source at home.

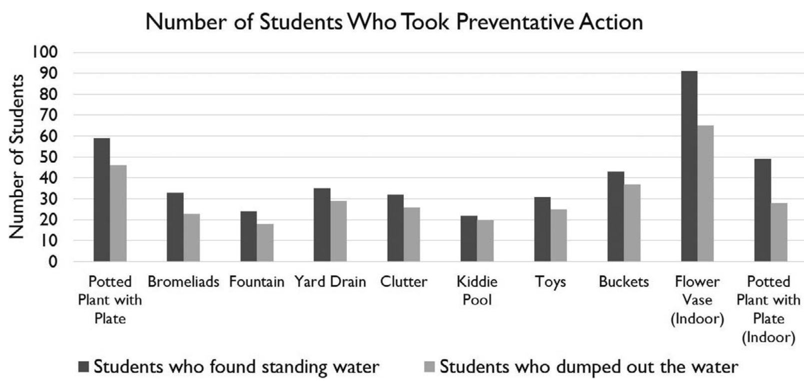

Figure 3.—Number of students who found and took preventative action with each type of larval habitat containing water.

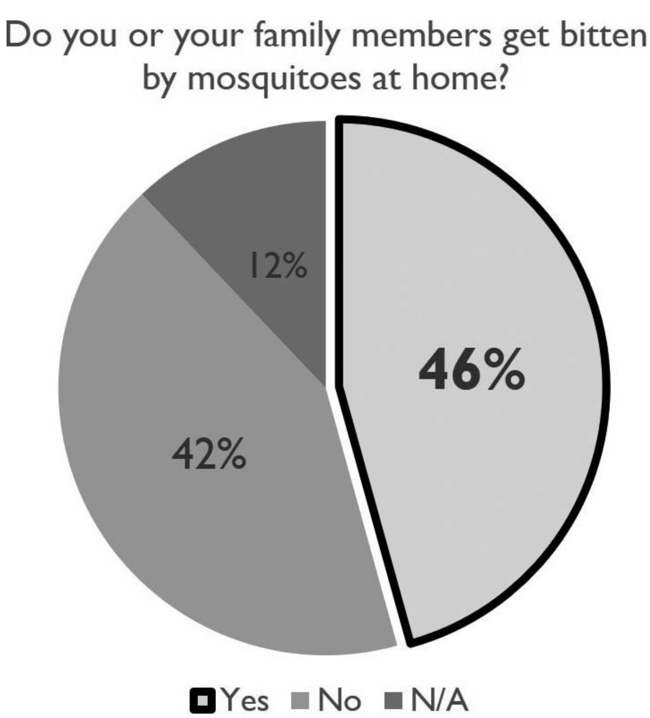

Figure 4.—Response to question (n = 116): "Do you or your family members get bitten by mosquitos at home?"

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

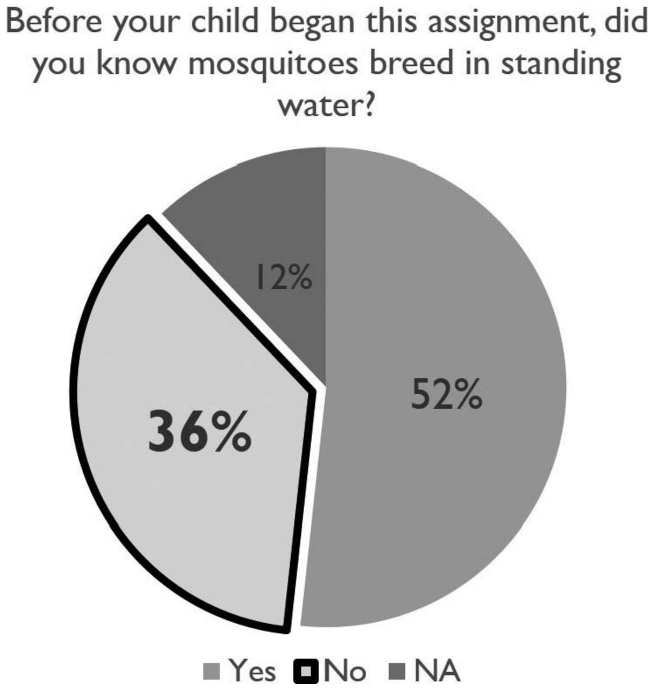

Figure 5.—Response to question (n = 116): "Before your child began this assignment, did you know mosquitoes breed in standing water?"

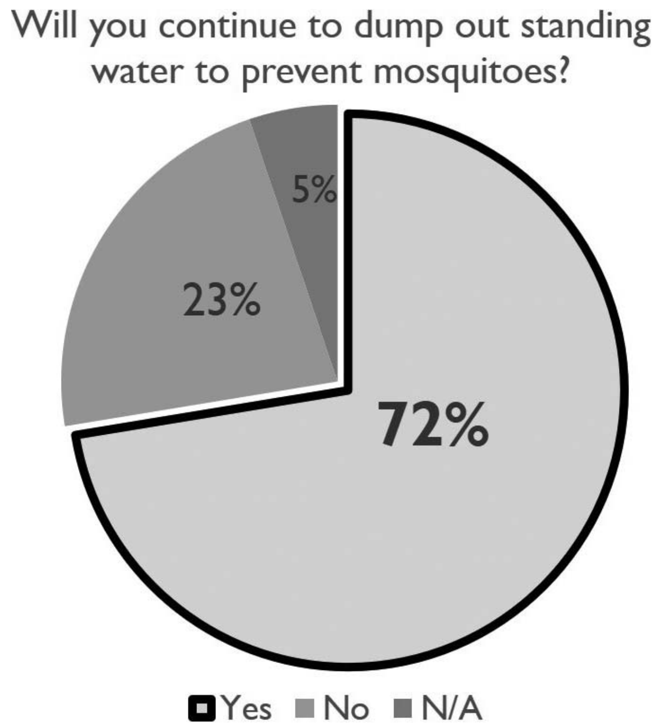

Figure 6.—Response to question (n = 116): "Will you continue to dump out standing water to prevent mosquitoes?"

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

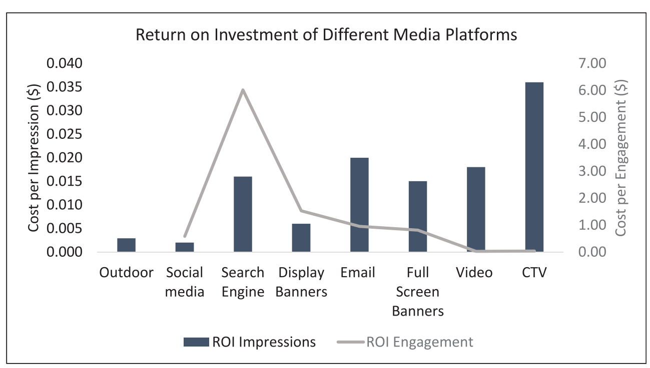

Figure 1.—Cost per view of media content (impression) and cost per website visit or video completion (engagement) for each media in the County of San Diego Fall 2019 Fight the Bitecampaign. ROI is return on investment.

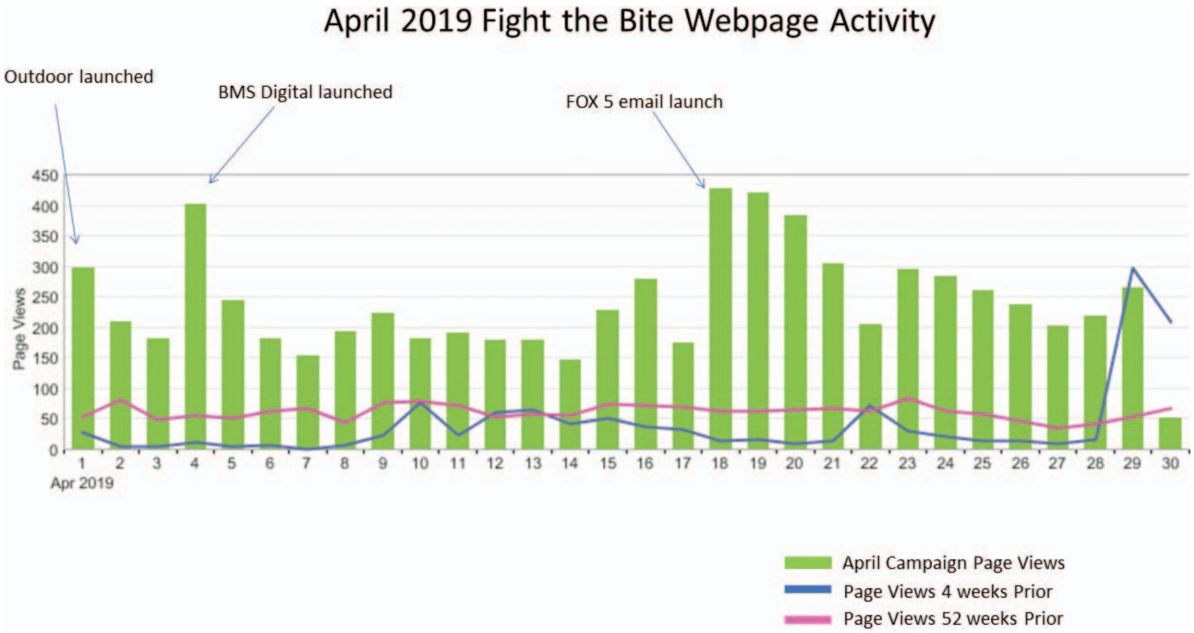

Figure 2.—Visits to the SDFighttheBite.com website during the County of San Diego Spring 2019 Fight the Bite media campaign. The blue line shows the number of website visits 4 weeks prior to the campaign. The pink line shows the number of visits 52 weeks prior during the 2018 campaign. Data compared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

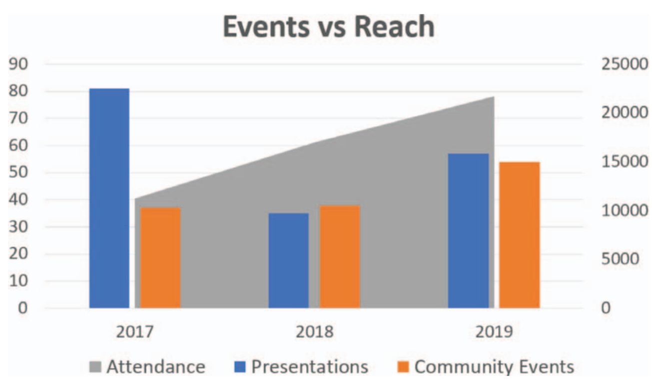

Figure 1.—The number of presentations and events the District participated in from 2017 through 2019The gray graph shows the gradual increase in number of people reached through these events. Despite attending less events in 2019 than in previous years, the District reached more residents than in previous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

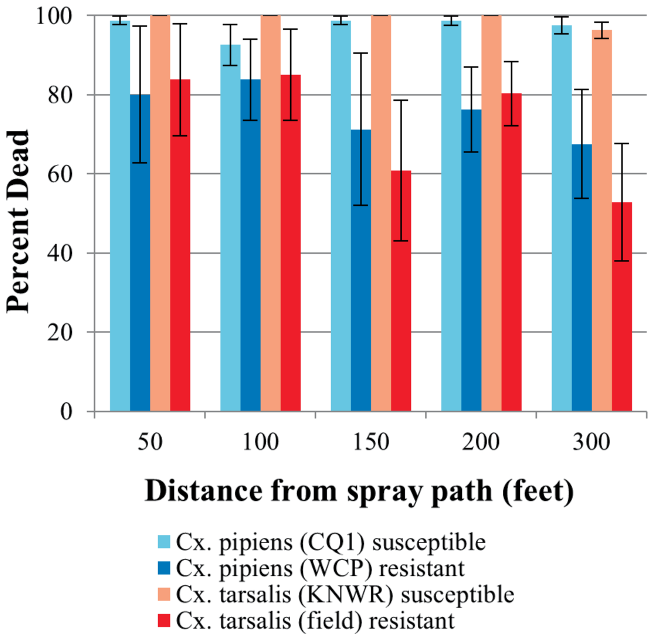

Figure 1.—Mortality in adult mosquitoes from 4 groups at 12 h post application in sentinel cages from a 2019 field evaluation of the adulticide Pyronyl Oil Concentrate #525 in Sacramento County, CA.

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.

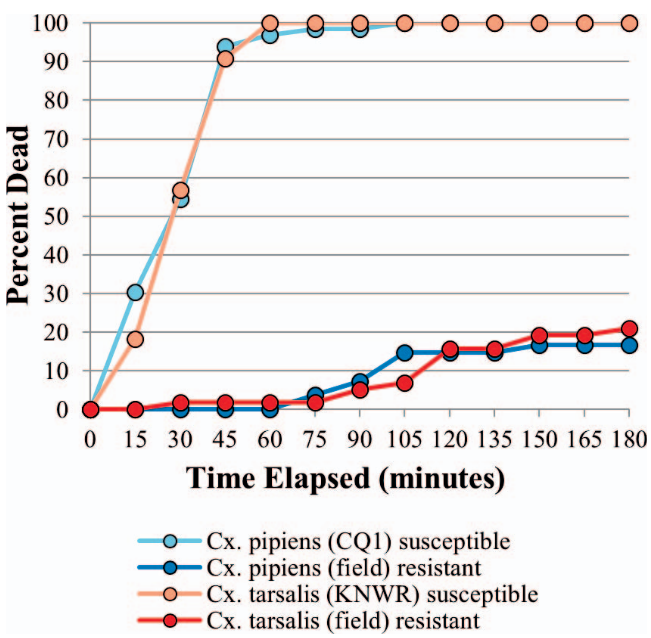

Figure 2.—Time mortality curves showing results of a bottle bioassay performed at the SYMVCD during the 2019 season. Two colony reared susceptible, and two resistant field-collected adult mosquito species were tested using a permethrin chemical standard.

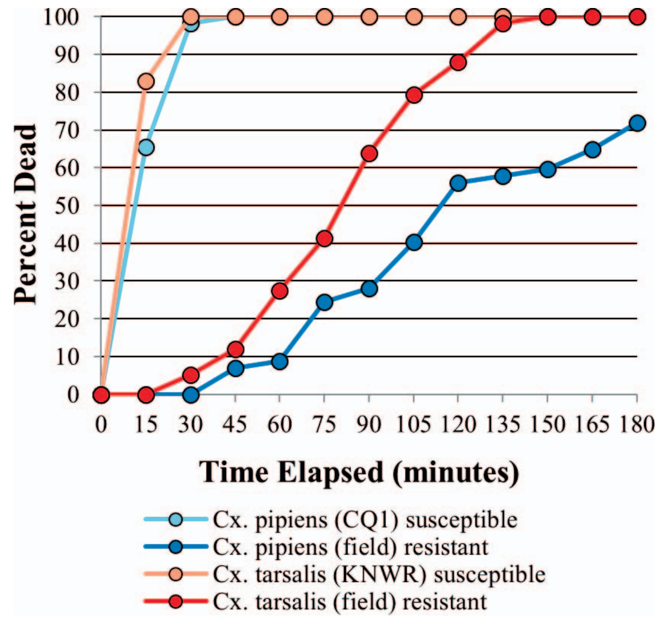

Figure 3.—Time mortality curves showing results of a bottle bioassay performed at the SYMVCD using a permethrin chemical standard combined with a piperonyl butoxide (PBO) chemical standard. These were tested against two colony reared susceptible and two resistant field-collected adult mosquito species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

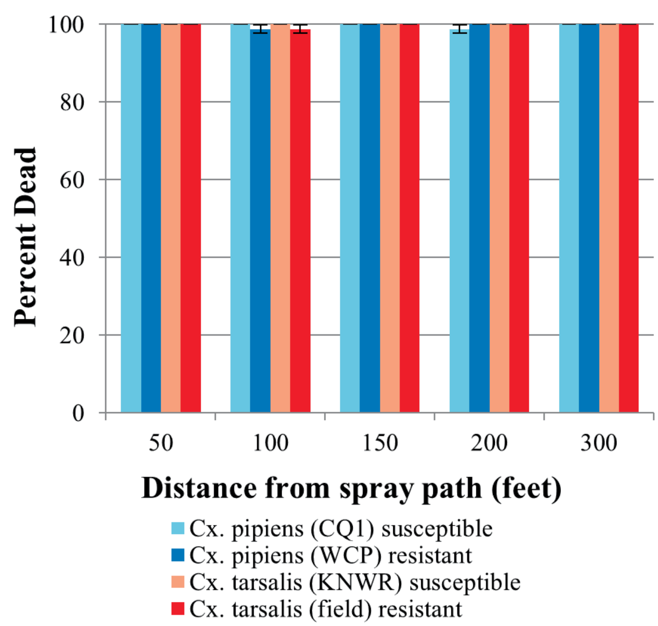

Figure 4.—Mortality at 12 h post application of four groups of adult mosquitoes exposed in sentinel cages during a 2018 field evaluation of the adulticide DeltAGard in Sacramento County, CA.

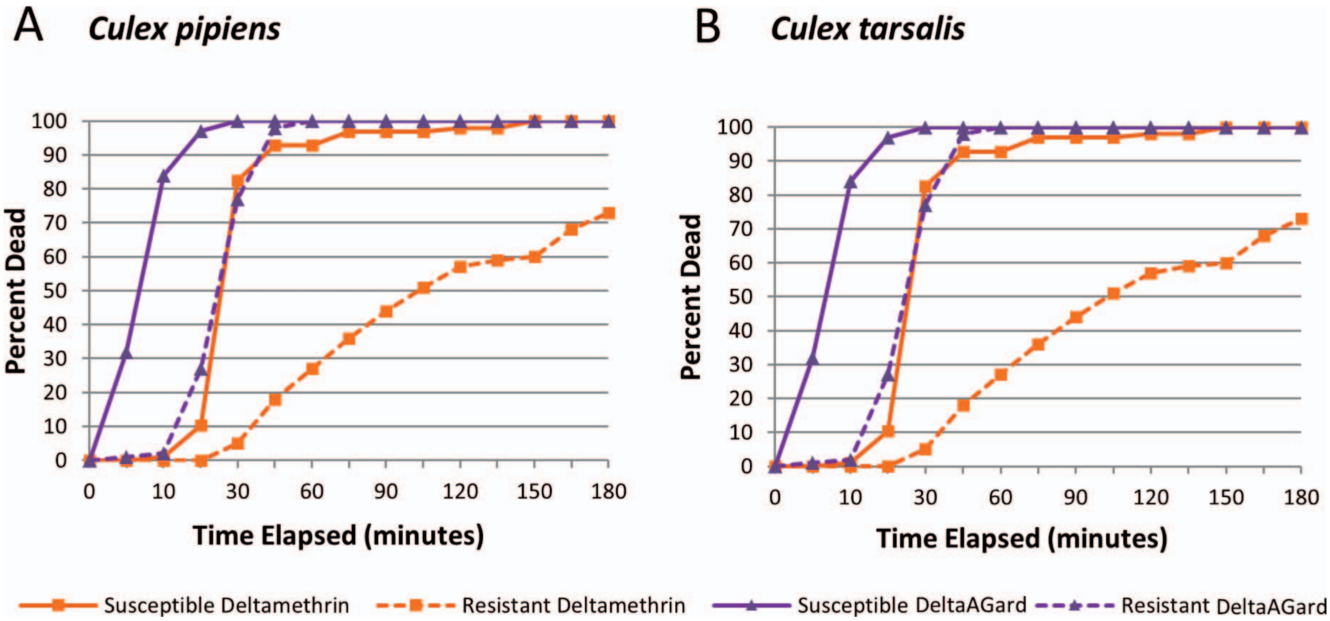

Figure 5.—Results of two separate bottle bioassays performed at SYMVCD designed to evaluate the presence of resistance in adult mosquitoes to the active ingredient deltamethrin. Chemical standard deltamethrin was compared to the formulated product DeltAGard. Test mosquito species represented in graph 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

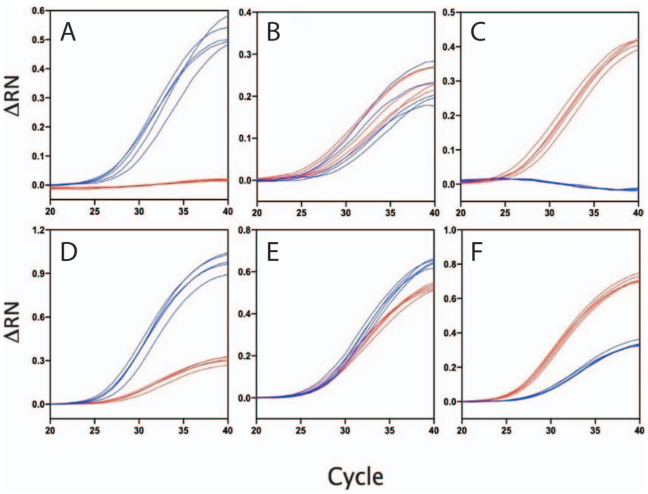

Figure 1.—Amplification plots (ΔRN vs Cycle Number) for *Culex pipiens* (A-C) and *Culex tarsalis* (D-F) with 5 representative specimens selected for each genotype. The blue and red lines represent amplification of the wildtype and mutant probes, respectively. (A) *Culex pipiens* homozygous wildtype;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

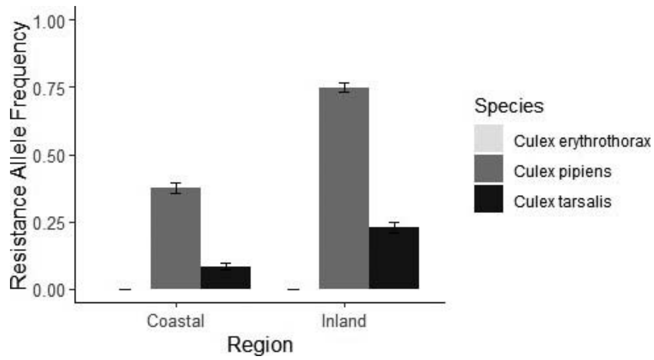

Figure 2.—Resistant allele frequency (F_R) by species and region. Light Grey, dark grey and black bars represent F_R for *Culex erythrothorax* (no resistance detected), *Culex pipiens* and *Culex tarsalis*, respectively.

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

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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

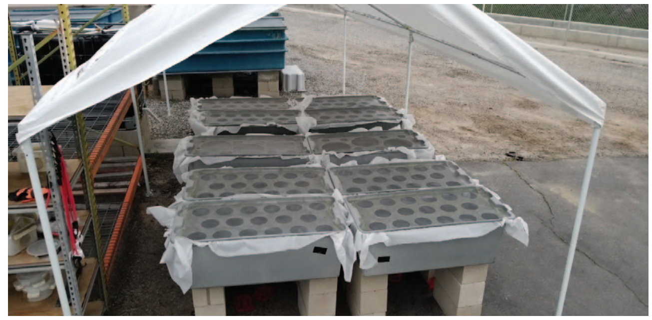

Figure 1.—Arrangement of ten medium-sized tanks (eight treatment tanks and two controls) for testing the efficacy of Sumilarv 0.5G against field and laboratory colony *Cx. quinquefasciatus* larvae.

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,

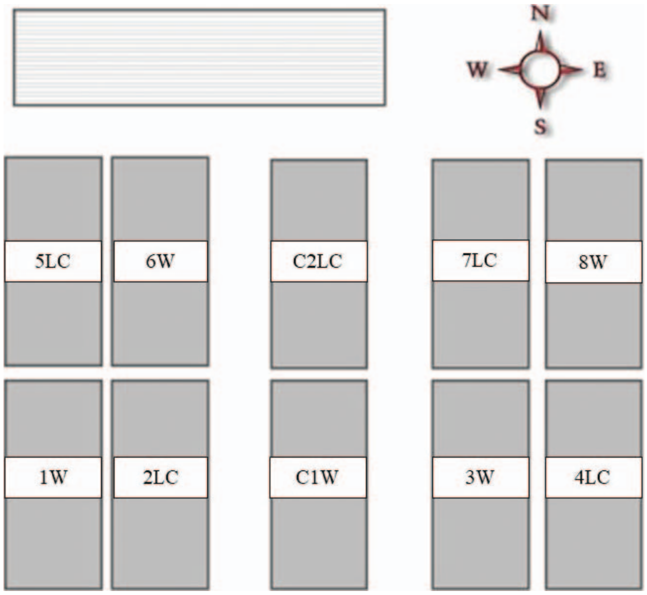

Figure 2.—Schematic of medium-sized tank block design. Tanks with wild (W) and laboratory colony (LC) *Cx. quinquefasciatus* larvae were alternated across rows with control tanks in the middle of the block.

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), ≤ 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-

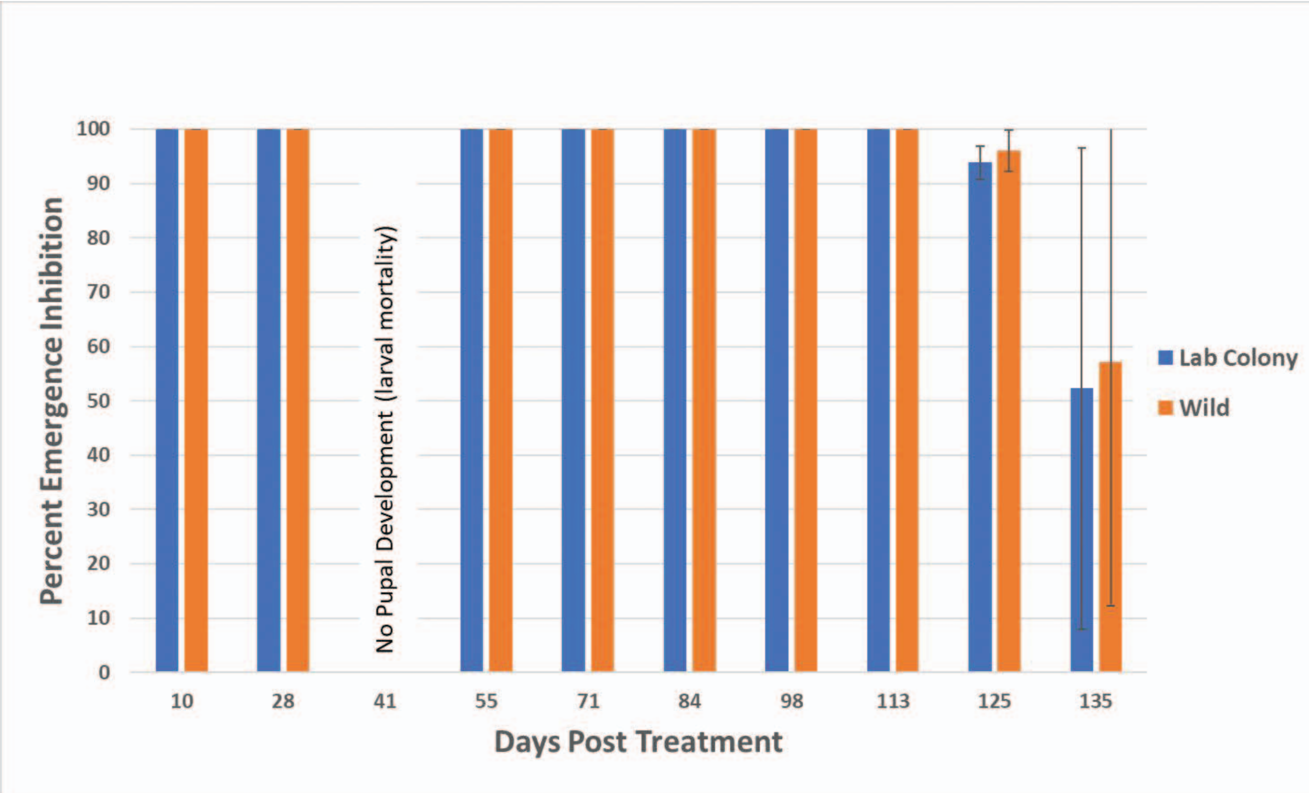

Figure 6.—Emergence inhibition of *Culex quinquefasciatus* after a single 10 g application of Sumilarv 0.5G in medium-sized (125 L) tanks. Mean ± 1 standard deviation (SD).

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 $\leq$ 30.5 cm (1 ft.) depth, 10 g is

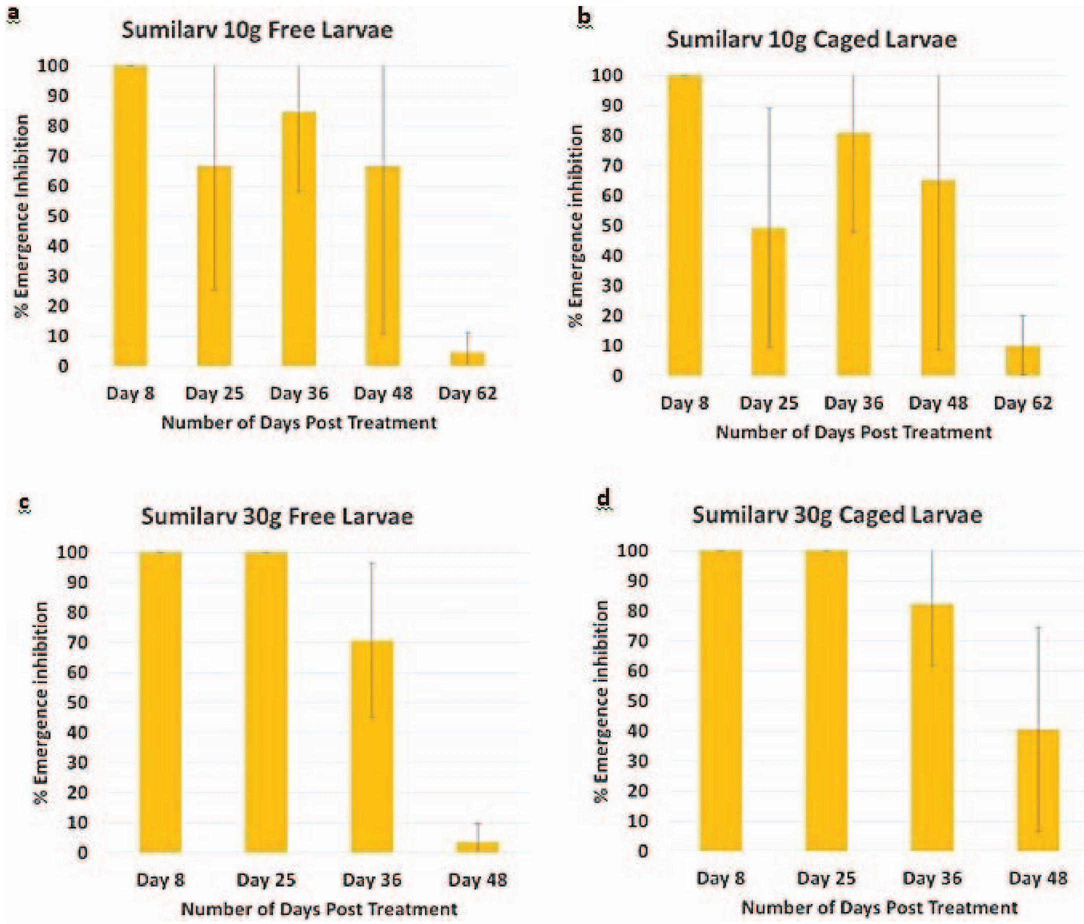

Figure 7.—Emergence inhibition for wild *Culex quinquefasciatus* larvae exposed to a single application of Sumilarv 0.5G in large tanks (1820 L) at applications rates of (a) 10 and (c) 30 g/tank with free-swimming larvae, and (b) 10 and (d) 30 g/tank of caged larvae. Mean ± 1 standard deviation (SD).

the recommended application; for water bodies 1892 L – 5678 L (500 – 1500 gal) and ≤ 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).

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The emergence of multiple *kdr* alleles in *Culex tarsalis* in California

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Introduction

In recent years, permethrin resistance has been identified in Northern California populations of *Culex tarsalis,* an important arbovirus vector in the western United States (CalSurv). Selection for insecticide-resistant *Cx. tarsalis* populations may be driven by exposure to insecticides from vector control as well as agriculture, but this has not been characterized. Here, we generated the first genome assembly of *Cx. tarsalis* and performed preliminary assessments of insecticide resistance allele frequencies of the well-characterize voltage-gated sodium channel mutation (*kdr*) in California's Central and Coachella Valleys between 2006 and 2018.

Methods

High-molecular-weight DNA extraction and PacBio sequencing of a single male *Cx. tarsalis* mosquito from the Kern National Wildlife Refuge (KNWR) strain first established from field collections in Kern County, CA in 2002 was performed at Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley. Genome resequencing library preparations were prepared with 25–50 ng of genomic DNA input, using the Nextera DNA Sample Preparation Kit and TruSeq dual indexing barcodes (Illumina). Amplicon-Seq was performed using gene-specific primers [e.g. at the knock-down resistance *(kdr*) locus in the *para* gene] with Illumina adapter tails.

Results and Discussion

The total HiFi *Cx. tarsalis* genome size was 800Mb (Quast v5.0.2) with an N50 of 58kb, the GC content was 36% and the largest contig was 757kb. Using whole-genome sequencing (WGS) and Amplicon-Seq on wild-caught *Cx. tarsalis,* we found that the susceptible kdr^{wt} allele was fixed in the Sacramento Valley counties of Sutter in 2006 (N=24 via Amplicon-Seq) and Yolo in 2008 (N=30 via WGS). Only a small sample was analyzed for Southern California, so results are inconclusive, but three specimens collected from the Coachella Valley in 2011 were purely susceptible (N=3). In more recent collections, we found that a recently established 2018 colony from Conaway, CA (Yolo County) was homozygous for *kdr*, including two distinct alleles: L1014F (TTT) and L1014S (TCA). Both *kdr* alleles also were detected in the San Joaquin Valley (Kern County) in 2018, but the 1014F was much more common than 1014S (62% vs. 4%, respectively via Amplicon-Seq of 6 pools of 50 individuals each). In the Coachella Valley in 2018, the 1014F *kdr* allele was detected at 50% allele frequency (N=8). Overall these data indicate that *kdr* increased substantially in *Cx. tarsalis* populations in California after 2006.

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Resistance risk assessment of microbial and insect growth regulator larvicides

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Abstract

Mosquito larvicides derived from microbial organisms and insect growth regulators have been increasingly used to control mosquito larvae worldwide. Their relative target specificity, non-target safety and environmentally friendly profile have been well documented. The current paper was intended to review and analyze the relevant information regarding resistance development and resistance management tactics. *Bacillus thuringiensis israelensis* de Bajac (*B.t.i.*) is a quick acting and highly target-specific biopesticide against mosquitoes, blackflies, and other nematoceran species. Resistance development against the intact complementary toxin complex of *B.t.i.* has been rare; however, low to high levels of resistance to individual toxins have occurred in laboratory mosquito populations. The toxins from bacterium *Bacillus sphaericus* Neide (recently renamed *Lysinibacillus sphaericus* Meyer and Neide) are also highly active against mosquitoes, toward which low to high levels of resistance have occurred in both laboratory and field mosquito populations. The Cyt1A toxin from *B.t.i.* and Mtx toxin from certain strains of *B. sphaericus* are the key components in resistance management to *B.t.i.* and *B. sphaericus*. Resistance management strategies have been well developed and implemented. Spinosad derived from *Saccharopolyspora spinosa* Mertz and Yao has been used recently for mosquito control; however, high levels of resistance and cross resistance have occurred in laboratory mosquito populations and management tactics have never been developed. Methoprene has been used to control mosquitoes for decades, and low to high levels of resistance have been occasionally reported in both laboratory and field mosquito populations. Studies on mechanisms and management of methoprene resistance are scarce. Very little attention has been paid to the resistance management in mosquitoes to other insect growth regulators such as pyriproxyfen and diflubenzuron. The prevention of resistance and restoration of susceptibility in mosquitoes to these biorational larvicides are crucial to the success of sustainable integrated mosquito management.

Insecticide resistance and the identification of resistance mechanisms in populations of *Culex tarsalis* from San Joaquin County, California

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Introduction

In the United States, one of the common vectors of West Nile and other encephalitis viruses is *Culex tarsalis*. This species generally is found in rural and agricultural habitats but in San Joaquin County, many of these environments are in close proximity to expanding suburban housing developments, putting the public at increasing risk. Although integrated vector management strategies are used to control this species, adulticide operations are an important component of minimizing the public health risk as they can provide the rapid elimination of infected adult females. The development of insecticide resistance threatens the efficacy of the adulticide products available for vector control but very little is known about the resistance status or mechanisms involved in this species. Our current study investigated pyrethroid and organophosphate resistance as well as target-site and metabolic resistance mechanisms in several San Joaquin County, California populations of *Cx. tarsalis*.

Methods

Mixed-age female *Cx. tarsalis* were field-collected from six areas in San Joaquin County, CA. To determine resistance status, bottle bioassays were used to compare field populations to the susceptible laboratory strain, Bakersfield Field Station (BFS), using glass bottles coated with diagnostic doses of permethrin, permethrin synergized with piperonyl butoxide (PBO), or naled; knockdown was recorded every 15 min for two hours. Colorimetric enzyme assays were performed to measure levels of the enzyme families α-esterase, β-esterase, mixed-function oxidase, glutathione-S-transferase, and acetylcholinesterase as well as test for insensitive acetylcholinesterase. A SYBR Green qPCR assay using allele-specific primers each with different length GC-rich tags and a melt curve of the resulting qPCR products was used to identify *kdr* mutations.

Results and Discussion

Varying levels of permethrin resistance were observed among the six San Joaquin County *Cx. tarsalis* populations, with mortality ranging from 0 to 26.0% at the 30-minute diagnostic time and 11.4 to 96.2% at the end of the two-hour assay. Mortality in all populations reached 100% when permethrin was combined with the oxidase inhibitor, PBO, demonstrating that oxidases are playing a role in permethrin resistance in these populations. L1014F and L1014S *kdr* mutations were found in all wild populations, with the F allele frequency ranging from 77.6-98.3% and S allele frequency ranging from 1.7 to 13.1%. The susceptible BFS strain did not have any *kdr* alleles, suggesting that the *kdr* mutations may have been a contributing factor in resistance in the field populations tested.

In the naled bottle bioassay, all but two populations reached >90% mortality at the 60-minute diagnostic time, and all but one of the populations reached 100% mortality after two hours. Esterases are known to be involved in organophosphate detoxification and the levels of α-esterases were highest in the two resistant populations, suggesting possible contribution to naled resistance development. One population also had significantly higher levels of acetylcholinesterase than all other populations which could contribute to the development of resistance to naled. No acetylcholinesterase insensitivity was detected in any of the San Joaquin County populations.

Conclusions

There was a wide range of permethrin resistance observed in the six San Joaquin County *Cx. tarsalis* populations, with oxidase enzymes appearing to be a contributing factor as demonstrated in the bottle bioassays using the synergist, PBO. The high frequency of *kdr* resistance alleles also indicated *kdr* may be a contributing factor. Most of the populations exhibited susceptibility to naled, but resistance may be beginning to develop in two populations due to significantly greater levels of *a*-esterase or overexpression of acetylcholinesterase. None of the San Joaquin County populations displayed acetylcholinesterase insensitivity.

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Truck- mounted ULV and larvicide equipment - inspection, maintenance and comparisons

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Abstract

Once SLEV- and WNV-positive mosquito samples are detected, the Laboratory and Operations departments of the Coachella Valley MVCD (District) work collaboratively to determine what plan of action will be taken to reduce mosquito populations and reduce the risk of virus transmission. The use of truck-mounted ULV equipment has been a key method in previous years, and recently, the addition of truck-mounted larvicide equipment has added to the methods available. Equipment inspection and maintenance is vital to maintaining a fully effective mosquito control program that can respond to the presence of virus-positive mosquitoes. We present herein truck-mounted equipment activities relating to pre-treatment setup, post-treatment inspection, flowrates, system flushing, filter inspection, circulating tank system, chemical awareness, system connections, and basic mechanical operations. Our information includes the use of GPS software via the Guardian Monitor. We highlight comparisons of truck larviciding equipment in our presentation, including the A1 Super Duty, the Curtis Dyna-Fog LV-8 and the Guardian 190L and contrast that with activities with truck mounted ULV equipment.

What Makes a Software Project Successful? Notes from the Field

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Abstract

Electronic data capture along with Web GIS saves money, increases efficiencies, ensures data quality, empowers decision making, and helps meet regulatory compliance. However, what makes the difference between a successful software project and an unsuccessful one? Herein, Frontier shares insights and lessons learned from other mosquito control and public health agencies who recently updated to Web GIS. Making the proper plans and involving the right people helps to ensure success. In this presentation we also show new developments pertaining to our office and field solutions for recording, mapping and reporting activities for all sizes of mosquito control agencies, including FieldSeeker software, mobile hardware, and drone technologies. We also introduce our new FieldSeeker GIS for Invasive Plant Control software which will be ready for beta testing for the 2020 field season.

Evaluating Dairy Lagoon Treatment Methods at the Sacramento-Yolo Mosquito and Vector Control District

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Abstract

The Sacramento-Yolo Mosquito and Vector Control District (District) tested the efficacy of several products in dairy lagoons during the 2019 mosquito season in an attempt to formulate an ideal treatment strategy for this difficult to control habitat. Historically, dairy lagoons produce a high abundance of *Culex pipiens* that affect communities situated in close proximity to them with both nuisance calls and West Nile virus amplification. Maintaining control of *Culex pipiens* in these dairy lagoons can be very time consuming with variable results depending on the product that is used. A new insect growth regulator, Sumilarv 0.5G, is labeled for dairy lagoons and was evaluated at two application rates this past summer. These lagoons were monitored on a weekly basis post treatment for duration of control and emergence inhibition and compared to our current treatment options. This presentation will show the results of what was observed in the field as these dairy lagoons were monitored throughout the trial period.

Aerial Evaluation of Pyrethrin products in Ultra Low Volume Applications over Rice Field Habitats

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Introduction

The Sacramento-Yolo Mosquito and Vector Control District (District) evaluated the efficacy of pyrethrin products in Ultra Low Volume (ULV) applications over rice fields targeting *Culex tarsalis* populations that have shown resistance in bottle bioassays performed by the District. The three different pyrethrin products tested included Pyronyl 525 Oil Concentrate (525), Pyronyl Crop Spray (Crop Spray) and Merus 3.0 which can be used over organic crops

Methods

The District conducted bottle bioassays to establish a baseline of resistance profiles in the *Culex tarsalis* populations from Yolo County areas being sprayed. The District utilized airplanes supplied through the District's aerial applicator service provider, Vector Disease Control International, that were calibrated for each product at the beginning of the year for the proper droplet spectrum and density recommended by the manufactures. Each of the three products were sprayed at the maximum application label dose to apply the same 0.002 lbs of active ingredient per acre for each product. Weather stations (Kestrel 5500 weather meter) were used to monitor temperature inversion and wind speed in the trial areas to compare the weather conditions in each block during the applications. A total of 10 sentinel cages were placed at 2,640 ft intervals along a 2 mile transect in each block: 5 cages contained 20 susceptible *Culex tarsalis* in each cage and 5 cages contained 20 wild caught *Culex tarsalis* from 5 different rice growing areas. Leading Edge spinners with teflon slides were set in the middle of each transect line. Slides were collected 1 hour after the spray was completed and were read using Drop Vision the next day to compare droplet spectrums and density. Crop Spray and 525 products were applied over blocks in the same geographical area separated by a 1.2 mile buffer zone and applications were started at the same time on the same nights. Both 525 and Crop Spray blocks were sprayed once a week for two weeks with the materials switched on the second week, so that each block was sprayed with both materials at least one time. Merus 3.0 applications were made over organic crops separate from the 525 and Crop Spray blocks, and three separate applications were made on three separate nights. Sentinel cages were observed for mortality 1 hour after application and then collected and observed for mortality again at 12 hours for each spray event.

Results

Applications using both 525 and Crop Spray resulted in an average of 60% mortality for wild populations when cages were observed 1 hour after spray and in over 80% mortality in wild populations when observed 12 hours after spray. The first Merus 3.0 application had weather conditions conducive to aerial application and resulted in a mortality average of over 60% in wild populations when observed after 1 hour and an average of 84.3% mortality in wild populations when observed after 12 hours. Two subsequent applications using Merus 3.0 in the same aerial block were not done because of unsuitable weather conditions. Based on the results from these trials, pyrethrin products produced effective control, with over 80% mortality against wild *Cx. tarsalis* populations that have demonstrated resistance in laboratory bottle assays. Further testing of Merus 3.0 in field trials will be forthcoming.

Acknowledgements

The District would like to acknowledge the generous assistance from Central Life Sciences, Target Specialty Products and Clark for their help in making this trial possible.

Discovery and management of *Aedes aegypti* in San Joaquin County, California, 2019

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INTRODUCTION

The yellow fever mosquito, *Aedes aegypti*, was first found in Madera and Fresno counties in 2013 and has since spread as far north as Merced County and as far south as San Diego County, California. San Joaquin County Mosquito and Vector Control District (The District) has been actively monitoring for this mosquito species since 2014 and has developed an *Aedes* Response Plan in the anticipation of its arrival. In the summer of 2019, *Ae. aegypti* was found in San Joaquin as well as in Stanislaus, Sacramento and Placer counties. This invasive mosquito

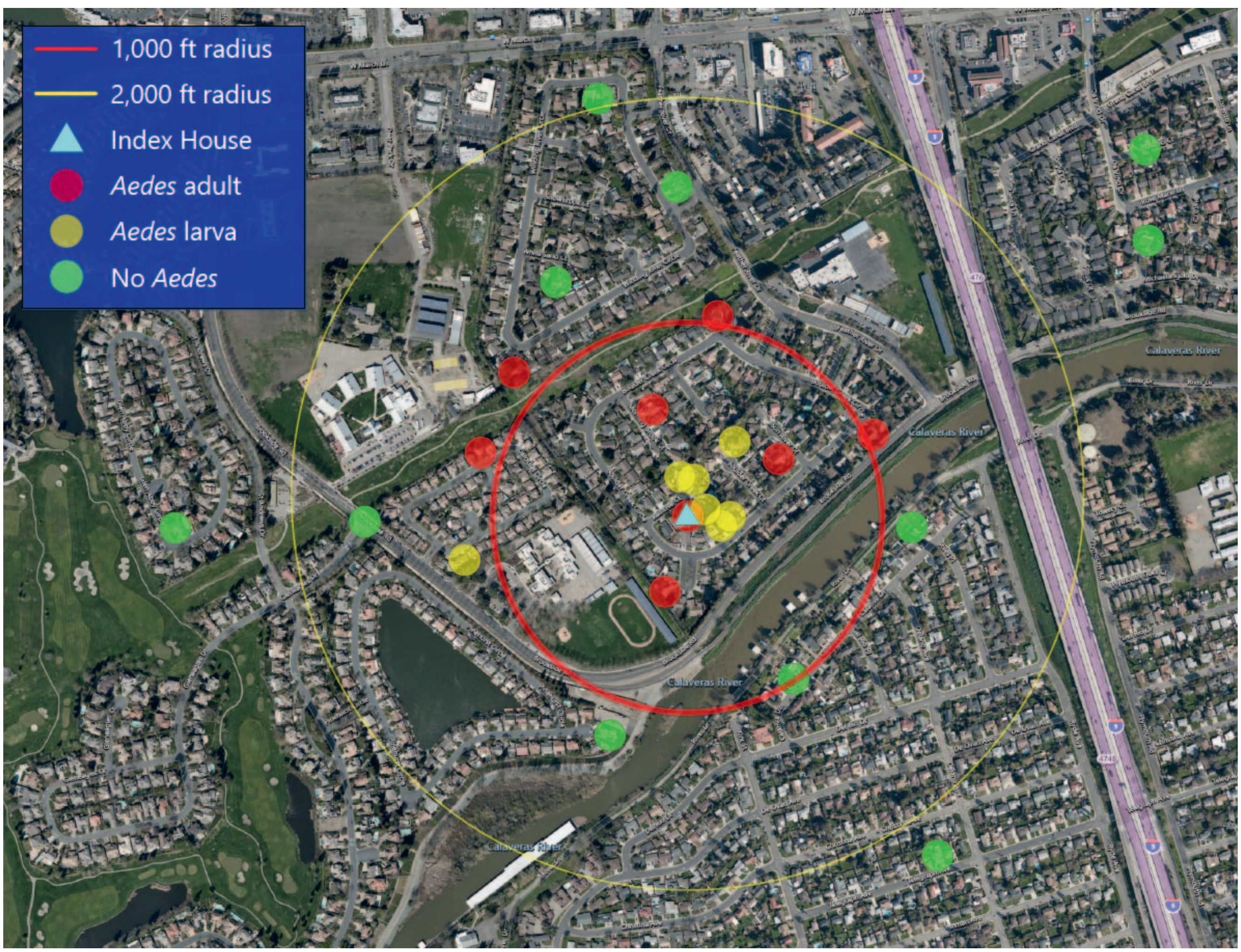

Figure 1.—Detection of *Aedes aegypti* in San Joaquin County. Shown are the index house of initial detection, subsequent larval and adult collections, and annuli used for sampling decisions.

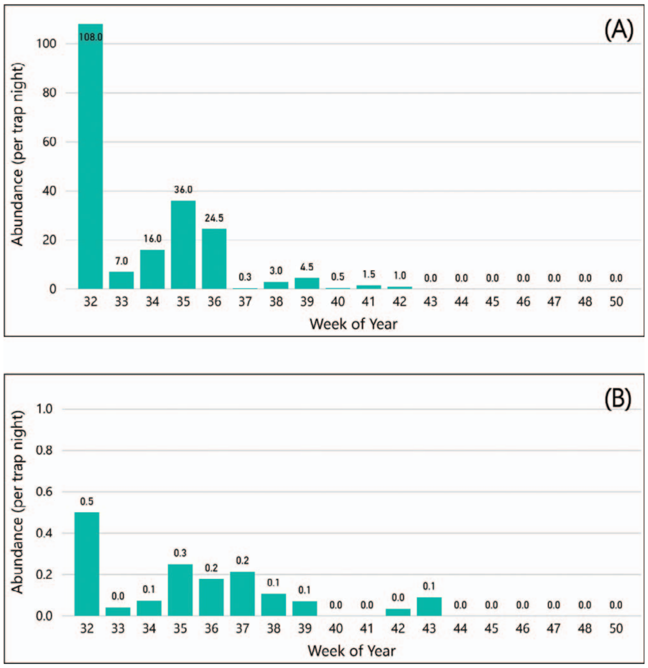

Figure 2.—Abundance of *Aedes aegypti* per trap night at the index house (A) and other trap sites (B)

species was initially found in response to a service request reporting "small aggressive daytime biting flies". It was located in a neighborhood west of I-5 and south of March Lane in the city of Stockton. The District responded immediately by following the *Aedes* Response Plan that prescribes Integrated Pest Management (IPM) strategies to combat invasive *Aedes* species. This paper describes the major specific measures used by the District and the control outcome of *Ae. aegypti*.

METHODS

Surveillance

To monitor the abundance and distribution of *Ae. aegypti,* initially seven BG-Sentinel and two CDC-AGO traps were placed within a ~1,000 foot radius range of the index house (37°58′39.71"N, 121°20′49.56"W). Subsequently, surveillance was expanded to a 2,500 foot radius using 18 BG-Sentinel and nine CDC-AGO traps. BG-Sentinel traps were baited with BG-lure and CO_2 and deployed for two nights per week. CDC-AGO traps were checked for adults weekly.

Source Reduction

The District initiated a door-to-door inspection campaign on the day when *Ae. aegypti* identity was first confirmed and continued the campaign throughout the season. This campaign was integrated with intensive public education either to facilitate property access or to directly involve residents in source elimination. All confirmed and potential breeding sources were either eliminated or treated primarily with Natular DT and tablets and occasionally with Altosid® briquets.

Adult Control

Ground ULV spray of DeltaGuard (Deltamethrin) was applied five times when the *Aedes* adult population was high or showed signs of rebound. At disease week 38, an aerial application of the organophosphate Dibrom (Naled) planned for West Nile virus vector control near the index house was expanded to cover the area with *Aedes* presence. The Dibrom application was started at 8:00 p.m. and finished at 9:00 p.m.

Larval Control

The primary larval control method was the wide area larvicide spray (WALS) of the Bti formulation VectoBac® WDG by using the A1 Super Duty mist sprayer. This sprayer produces a Bti-containing mist that can drift to cryptic, difficult-to-reach sources. To verify the material drift, 12 ounce plastic cups were set at 21 locations with different vegetation cover. Locations included the front yard, back yard and side yard. Cups were brought back to the laboratory and 25 field collected *Culex pipiens* 2nd-3rd instar larvae were introduced to each cup to evaluate spray penetration using larval mortality.

The District also used 12 In2Care traps at the index house and its surrounding area to supplement the WALS application. In2Care traps are loaded with the insect growth regulator pyriproxyfen. Gravid females are attracted to In2care traps, coated with pyriproxyfen, and deposit pyriproxyfen into the next breeding source they visit. One to two In2Care traps were set per house 50-100 feet apart. Similar to the WALS application, ovicups were deployed at week 39 to verify trap efficacy. Three ovicups were set at each of the five sites that were about 30-50 feet away from the In2Care traps. Ovicups were left in the field for 4 weeks. There were no other larvicide applications during this period. Ovicups were brought back to laboratory and 25 field collected *Cx. pipiens* 3rd-4th instar larvae were introduced to each cup to evaluate adult emergence rate.

In addition, three PG&E utility vaults near the index house were treated with Natular G30 granule at week 39.

RESULTS AND DISCUSSION

Surveillance

The first week surveillance data showed that *Ae. aegypti* adults were found as far as 600 feet away from the index house. The abundance was 108 adults (both males and females) per trap night at the index house and 1.5 adults per trap night at the other two trap sites where *Aedes* was found (Fig. 2). Property inspections located six breeding sources, which were within 600 feet from the index house. The surveillance data at 15 weeks showed the distribution of *Ae. aegypti* was limited to a range slightly larger than a 1,000 foot radius, and that the majority of the adults were collected at the index house (Fig. 1, 2). No adults were found after 12 weeks of intensive *Aedes* control.

The surveillance data from the first few weeks showed that *Aedes* had a limited distribution and that the majority

Figure 3.—Property Inspection Status with the community near the index house.

of mosquitoes were found at the index house. This offered a good chance to contain the *Aedes* population and its spread, although eradication of them may not be realistic. With this information, we used as many control methods as possible in the area.

Source Reduction

Source reduction has been reported to be one of the best methods to combat invasive *Aedes*, and our District allocated considerable time and resources to conduct door-to-door inspections. We located a few breeding sources, which included plant saucers, drains, vases, ornamental ponds, discarded cups and a Jacuzzi tub. However, we were only able to access 61.6% of the properties after eight weeks. The property inspection map shows we inspected most of the properties close to the index house, but we could not access many other properties in nearby areas (Fig. 3). We discovered seven breeding sources in the first three weeks and none thereafter. Evidently, not all sources could be located by property inspection and other methods were needed to treat unfound sources.

Adult Control

Ground ULV application had a large impact on *Aedes* population. When *Aedes* abundance was at the highest level just after first discovery, the first two Ground ULV applications reduced the *Aedes* population by 93.5% (Fig. 4). The first ground ULV of DeltaGuard (Deltamethrin) was conducted on Friday of week 32 and the second on Monday of week 33. BG-sentinel traps were running from Tuesday to Thursday for week 33. The spray right before trapping nights clearly showed application efficacy (Fig. 4). The third ULV application was conducted on Friday of week 33. The following week abundance data showed the population actually rebounded. This suggested that there was continued adult emergence. The fourth and fifth spray were both applied on Fridays. The following week abundance data showed the population was reduced by 98.8% and 88.9%, respectively (Fig. 4). Regarding the

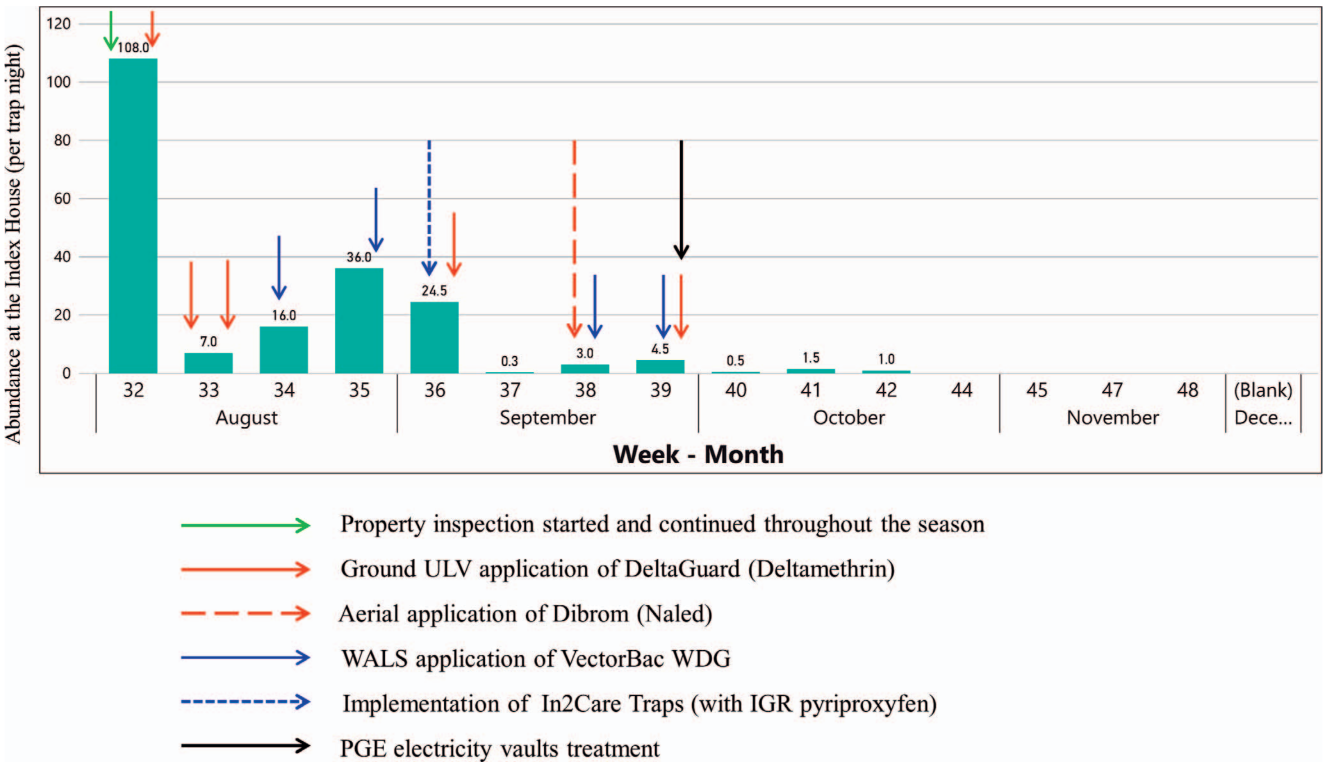

Figure 4.—Timeline of *Aedes aegypti* management activities and abundance per trap night at the index house.

aerial application of organophosphate Dibrom (Naled), it was convenient to expand the spray zone for WNV vector control to cover the *Aedes* area. Because Dibrom was sprayed at night, it was suspected that *Aedes* might not have been active and sufficiently exposed to the pesticide. This will be evaluated in the next season.

Larval Control

Mortality in plastic cups to verify the WALS application of VectoBac® WDG ranged from 0% - 100%, and did not correlate with location and vegetation coverage. Three cups in wide-open locations in the front yard had zero mortality, whereas two cups under bushes in the back yard had 98% mortality. This result is not consistent with the same study performed by the Coachella Valley Mosquito and Vector Control District, in which mortality was more than 90% in all cups. Our application area is a well-established residential neighborhood with extensive landscaping. There are plenty of trees ranging from 15 to 50 feet tall, which can disrupt the spray mist creating nonuniform drift movement. Secondly, the weather condition could play a role. When the WALS application was conducted, the wind speed was less than 2 mph and insufficient to assist spray drift.

The In2Care trap verification results were promising. Ovicups at three sites had an emergence rate of 75 to 90%, which suggested there was no pyriproxifen in the cups (Fig. 5). All three ovicups at site one had a 10% to 25% emergence for the first batch of *Cx. pipiens* larvae. After 17 days, the entire first batch of *Cx. pipiens* larvae were dead, and a second batch of 25 larvae were introduced, which resulted in an emergence rate of 5 to 16.6%. Similarly, the emergence for ovicups at site three was 0% to 5% for both batches of *Cx. pipiens* larvae. Emergence data suggested ovicups at site one and three had chemicals preventing mosquitoes from emerging. This indicated that In2Care traps worked. This is interesting because In2Care traps rely on gravid females to disperse pyriproxyfen, but *Aedes* abundance at the index house area was less than two mosquitoes per trap night during the entire time ovicups were deployed.

From weeks 37 to 39, abundance from BG-sentinel traps (excluding the one at the index house) was continuously decreasing, whereas abundance at the index house was increasing. At week 39, three PG&E utility vaults in front yard near the index house were treated without verification of *Aedes* breeding due to access issues. Coincidently, abundance at the index house dropped noticeably thereafter.

CONCLUSIONS

We were able to quickly reduce the population level, maintain suppression to <1 mosquito per trap night for eight weeks, and eliminate all *Aedes* mosquitoes in traps during the following 11 weeks. *Aedes* distribution did not appear to expand further than a 2,000 foot radius from the index house. Overall, the *Aedes* Response Plan worked

Figure 5.—Distribution of ln2Care traps and monitoring ovicups in relation to the index house.

effectively and we will continue to use and improve it based on new knowledge learned from our County and elsewhere.

ACKNOWLEDGEMENTS

The authors would like to thanks all the employees in San Joaquin County Mosquito & Vector Control District who participated in *Aedes* management. Their dedication made *Aedes* management a success. Special thanks to Andrew Provencio for taking the lead in *Aedes* trapping, Keith Nienhuis, Julian Ramos and other technicians in property inspection, Aaron Devencenzi in public outreach, John Fritz and Eddie Lucchesi in operational and program management, and other field technician in chemical application.

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Mosquito control efforts in duck club habitats in the Coachella Valley

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Abstract

Every season during fall to early spring, the Coachella Valley MVCD focuses extensive time and effort into controlling large, man-made duck club habitats. The large acreage of water, the constant inflow of new water and flooding practices result in large numbers of larval mosquitoes produced from many ponds. These mosquitoes transmit West Nile virus and St. Louis encephalitis virus from summer into the fall, and keeping populations low is critical to reducing virus transmission the following year. Herein, we review planning procedures for optimizing work at the duck clubs during a busy mosquito season. This talk includes designing a logistics plan that includes communication with property managers, control product selection that includes rotation practices for resistance management, assignment of shared equipment, delegation of work, and data collection. Upon returning from the field, we review sampling procedures and best treatment practices to ensure that supervisors know the scope of the labor needed during treatments and inspections. Workloads are reviewed weekly to accommodate the inspections and the changing environment.

It Takes an Emergency to Learn What You are Not Prepared for: Evolution of Orange County Mosquito and Vector Control's Urban Adult Mosquito Control Program

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Abstract

In 2014 Orange County experienced an unexpected and severe West Nile virus (WNV) epidemic and was the jurisdiction in the nation with the highest number of human infections (278 infections and 8 deaths). At that time, the Orange County Mosquito and Vector Control District (OCMVCD) was ill prepared to handle an arbovirus disease emergency of this magnitude. Although OCMVCD maintained an extensive larval mosquito control program, adult mosquito control applications were largely reserved for nuisance mosquitoes in coastal areas (~ 1% of treatments). OCMVCD's response to earlier modest WNV outbreaks in 2004, 2008, and 2012, only included enhanced mosquito and dead bird surveillance, shortened cycle times for control of larval sources, and expanded public education efforts. An attempt to target WNV activity hotspots with truck-mounted adult control applications in the highly impacted city of Santa Ana in 2014 was met with public opposition from the city. In the five years following the 2014 outbreak, OCMVCD has improved and expanded its adult mosquito control program to mitigate WNV activity in urban areas. Some of the program developments include: pesticide efficacy studies, added equipment (backpack and truck-mounted fogging units, and calibration testing technology), a new position to oversee pesticide management, conducting aerial readiness steps, adopting a supplemental response plan for WNV emergencies, identifying high risk cities for risk communication to the public/stakeholders, creating a door-to-door team for area-wide inspections and education in affected areas, multiple emergency management exercises and training, improving messaging and notification for areas targeted for urban adult control, coordination with agency partners, and implementing a risk-based surveillance grid designed to prioritize and expedite operational responses. These program enhancements have successfully supported OCMVCD's efforts to mitigate WNV activity and other arboviral disease threats in urban high risk areas, helped gain public and political acceptance and support for adult mosquito control, and served to better prepare the agency to prevent and protect against the next epidemic.

Genomic epidemiology of West Nile virus in California

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Introduction

Since its introduction in 1999, West Nile virus (WNV) has become endemic in the United States and has caused 45,000 confirmed human cases and 2,000 deaths. The virus causes localized outbreaks in the summer but the size and timing of these outbreaks is heterogeneous within and across different states and counties.

Methods

To investigate the drivers and dynamics of seasonal WNV outbreaks, we partnered with vector control and other public health laboratories across the United States and started the WestNile4K (https://westnile4k.org/) project. As the first phase of the project, we sequenced over 700 genomes of WNV with high spatio-temporal sampling from dead birds and *Culex* mosquitoes across three longitudinally dispersed counties in California: Sacramento-Yolo, Kern and San Diego. We analyzed this genomic dataset along with epidemiological data in a Bayesian phylodynamic framework to elucidate transmission patterns and covariates of the spread of WNV.

Results and Discussion

We reconstructed the time-resolved phylogeny of these genomes using Bayesian phylogeographic analyses. Given the high spatio-temporal sampling we were able to show that the virus was able to overwinter in northern, central and southern California over multiple years (Fig. 1).

However, there were a few viral lineages that moved between the three regions as shown in Fig. 2A. We applied a similar methodology to elucidate the transmission of the virus on a localized scale in three cities: San Diego (San Diego county), Bakersfield (Kern county), and Davis (Yolo county). Fig. 2B illustrates the movement of the virus in San Diego county and shows how the central-city region

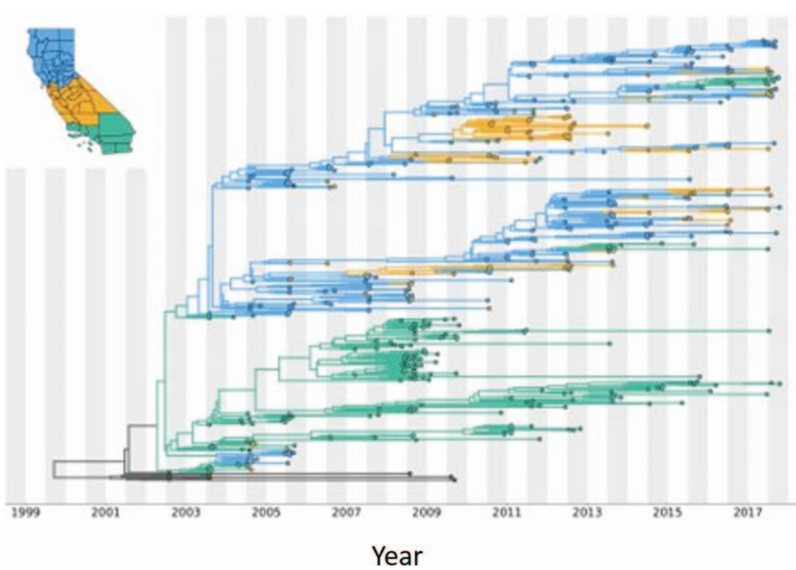

Figure 1.—Reconstructed time-resolved phylogeny of West Nile virus genomes sequenced in California. Internal nodes are colored by inferred location. Tips are colored based on sampled location and correspond to location colors on the inset map.

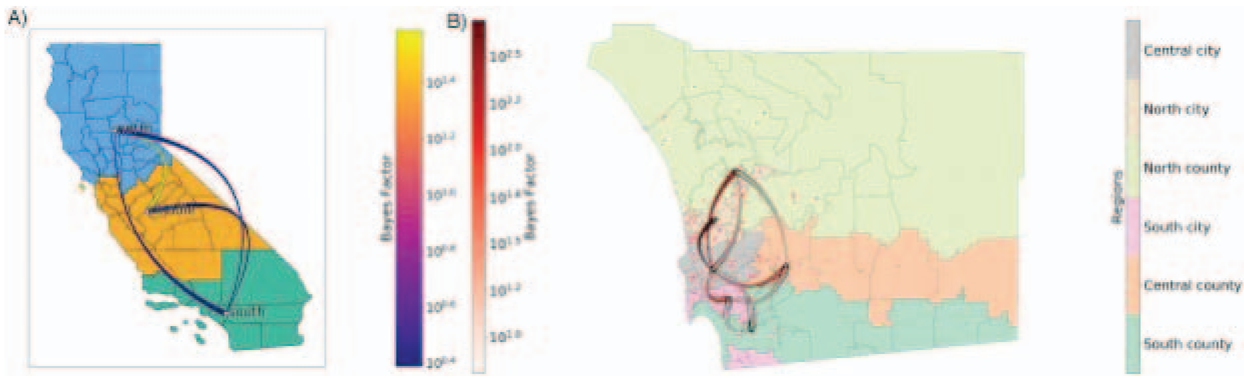

Figure 2.—A) Transmission patters across California that were selected using Bayesian stochastic search for variable selection (BSSVS). Thin end of the bezier curve represents the source and the thicker end represents the destination. Curves are colored based on Bayes factor support. B) Transmission patterns across San Diego county that were selected using BSSVS. Thin end of the bezier curve represents the source and the thicker end represents the destination. Curves are colored based on Bayes factor support.

acts as a source and seeds viral lineages into other parts of the county.

In addition to elucidating transmission patterns we used phylodynamic analyses to assess the contribution of environmental and ecological factors such as temperature, precipitation, vegetation and mosquito abundance to the spread of the virus in California.

Conclusions

Our study shows how genomic epidemiology can be used on a county-level and state-level to investigate the drivers of mosquito borne viral outbreaks and develop effective mitigation strategies.

Effects of storage temperature and freeze-thaw cycles on the stability of West Nile virus positive mosquito homogenate in the Ambion® MagMAX^{TM} lysis/binding solution

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INTRODUCTION

Storage conditions are crucial factors for chemical reagents and laboratory samples. The stability of viral nucleic acids in buffer solutions can vary based on the chemical used and storage conditions (Fleige and Pfaffl 2006, Huang et al. 2017). The goal of our study was to enhance our understanding of the stability of West Nile virus (WNV) positive mosquito homogenate in the Ambion® MagMAX^{TM} lysis/binding solution based on five storage conditions: storing samples in -80°C and -20°C freezers, a +4°C refrigerator, a +23°C room temperature environment, and after multiple freeze-thaw cycles.

METHODS

Twenty-seven WNV positive mosquito pools from 2017 were combined into one 50mL conical tube. Each mosquito pool had 500µL of retrievable homogenate, and a total of 13.5mL of homogenate was accumulated. The mean cyclic threshold (C_T) value of the mosquito pools used was 22.2 (95% confidence interval 21.2-23.1). The combined homogenate from the 50mL conical tube was aliquoted into five sets of 26 tubes. Each set was stored in an enclosed box and placed in the corresponding storage. One sample set each was stored in the -20°C freezer, +4°C refrigerator, a +23°C room temperature environment, and two sets were stored in the -80°C freezer, where one set underwent four freeze-thaw (F-T) cycles from the -80°C freezer to room temperature in 1.5 hours, every week throughout the project period. Viral RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT-PCR) kits were from Life Technologies. Aliquoted samples were extracted using the MagMAX^{TM} Express Magnetic Particle Processor (24 well) with the MagMAX^{TM}-96 Viral RNA Isolation kit. Quantitative RT-PCR was performed in triplicates using the TaqMan^{TM} Fast Virus 1-Step Master Mix. Samples for this study were simultaneously tested four times a week with the District's routine triplex testing for WNV, St. Louis encephalitis virus, and Western equine encephalitis virus to reflect a realistic laboratory work environment. The C_T values were averaged weekly and these values were used to calculate C_T differences and variations of each sample set (Stephenson and Warnes 1998, Watzinger et al. 2004, Merrill et al. 2012, Burkhalter and Savage 2017). All samples were tested on the first week and then regularly on the 13th week, because the same study was conducted in 2018, but it only lasted up to week 13 [where WNV remained detectable] due to budgetary constraints. Additionally, storage temperatures were measured three times daily and were averaged per week. Weekly mean C_T values for each sample set were also compared to the -80°C sample set using multiple paired t-tests, since storing samples in the -80°C freezer is the gold standard storage condition.

RESULTS AND DISCUSSION

RNA within the WNV positive mosquito homogenate remained detectable under all five storage conditions for 18 weeks and 64 F-T cycles. There was a total of seven weeks where samples for this study were tested, which included week 1 and weeks 13 through 18. As for storage temperatures, all, except the -80°C freezer, were three degrees lower than expected (Fig. 1). Compared to the initial mean C_T reading shown in Table 1, C_T values dropped an average of 3.5 values (Fig. 2). However, weekly

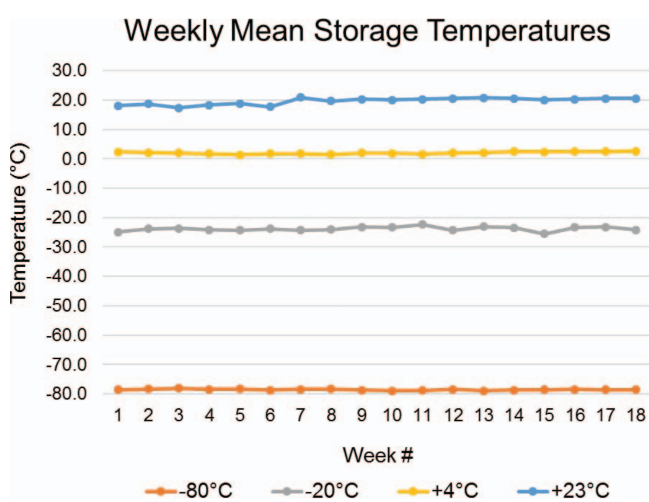

Figure 1.—Weekly means of storage temperatures measured three times each day. All recorded temperatures, except for the -80°C freezer, were three degrees lower than expected.

Table 1.—Initial mean C_T values taken on the first week of the project. These values were used to calculate the weekly mean C_T difference of each sample set.

Initial Mean CT Values (Week 1)	
Storage Condition	Ct. Value
-80C	27.7
F - T	26.9
-20C	28.7
+4C	28.9
+23C	28.4

mean C_T values did not differ >2 C_T from week 13 through 18. The remaining extraction reagents from the previous year were used for the initial testing on the first week of the study, whereas, that newly prepared reagents that were used on week 13 enhanced RNA extraction efficiency and resulted in a large decrease in C_T values.

Paired t-tests on the weekly mean C_T values between each sample set and the -80°C sample set showed a significant difference only between the -80°C and F-T sample sets (Table 2, df=6, P=0.003). Notable variation in C_T values was seen in both the -20°C and +23°C sample sets along with high C_T readings (Fig. 3). These data were expected for the +23°C conditions as these samples were being stored in an environment with elevated temperatures. The -20°C samples were difficult to handle, because the homogenate was viscous once fully thawed at room temperature. Even though the +4°C sample set had the largest drop in C_T value from its initial reading, it had consistently lower C_T values compared to all other sample sets. The +4°C sample set remained in the same liquid state throughout the study while being stored in a chilled environment. Variations in C_T values could have been attributed to several factors, such as large amounts of RNase introduced to the samples when multiple mosquito pools were combined and the quality of buffer solutions.

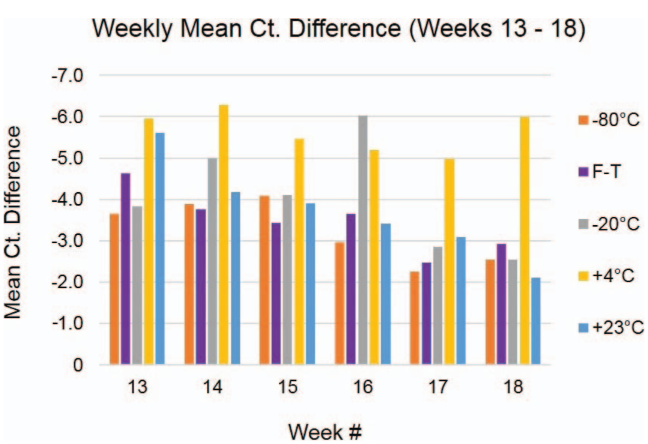

Figure 2.—Weekly mean C_T differences of each sample set. Difference was calculated by taking the mean C_T value of a sample set on a specific week minus the initial mean C_T value for the corresponding sample set. C_T values dropped an average of 3.5 from the initial reading due to fresher extraction reagents used on weeks 13 through 18.

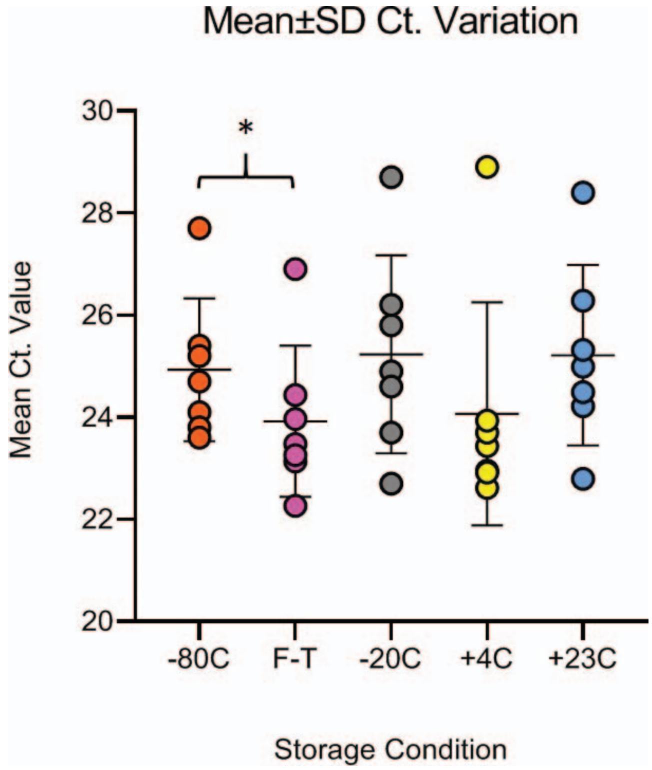

Figure 3.—Variations in weekly mean C_T values, with standard deviations, of each sample set (-80°C, mean=24.9, SD=1.4; F-T (Freeze-Thaw), mean=23.9, SD=1.5; -20°C, mean=25.2, SD=2.0; +4°C, mean=24.1, SD=2.2; +23°C, mean=25.2, SD=1.8). The highest C_T value readings were from the first week of the project and the outliers of the chart. * denoted significance by t-test at 0.05 probability level.

CONCLUSIONS

As WNV remained detectable in mosquito homogenate in the Ambion® MagMAX™ lysis/binding solution for 18 weeks under five different storage conditions, this study indicated that surveillance programs that do not have an ultralow temperature freezer readily available may be able to store and later test field specimens. Future work on virus titer measurements, comparing buffer solution stability under different storage conditions, and replicating this study on other viruses will help provide a better understanding of viral degradation (Livak and Schmittgen 2001, Turrell et al. 2002, Griffiths et al. 2011, Ryba et al.

Table 2.—Multiple paired t-tests comparing weekly mean C_T values to the -80°C sample set. A significant difference was found only between the -80°C and freeze-thaw sample sets at a 0.05 probability level.

Mean Ct. Values Compared to -80C Sample Set (df+6)		
Comparisons	P-Value	Significant Difference
-80C vs F-T	0.003	Yes
-80C vs -20C	0.521	No
-80C vs +4C	0.082	No
-80C vs +23C	0.383	No

2012, Andrews and Turell 2016). If a laboratory does not have access to ultralow temperature freezers, or has been experiencing freezer breakdowns, our study indicated that viral samples can be stored in lysis buffer under non-optimal conditions and remain positive for at least four months.

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There are autogenous *Culex pipiens* in our catch basins, should our District be concerned?

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Abstract

The Sacramento-Yolo Mosquito and Vector Control District (District) previously identified autogenous *Culex pipiens* breeding in catch basins within our service area. Based on the species description, autogenous *Cx. pipiens* are considered form *molestus*. However, genetic intermixing of *Cx. pipiens* form *pipiens*, *Cx. p*. form *molestus*, and *Cx. quinquefasciatus* is possible, therefore understanding this species complex is difficult. Independent of a precise species designation, it is important to understand whether these mosquitoes require special operational considerations for control. Multiple urban catch basins were sampled to determine the prevalence of autogeny in these populations and a colony was established to assess the adult and larval insecticide resistance status of this autogenous population. Bottle bioassays indicated that our form *molestus* colony had elevated resistance to permethrin and deltametrin, but not naled, when compared to susceptible controls. The form *molestus* colony was not significantly different from two other urban field-collected *Cx. pipiens* populations that were not assessed for autogeny and represent a baseline for insecticide resistance in the area. Therefore, in the Sacramento area control strategies that work to control mosquitoes at the baseline levels of resistance will most likely be effective against form *molestus*.

Introduction

Culex pipiens L. and *Cx. quinquefasciatus* Say are not native to North America and were introduced well before the establishment of mosquito control programs. It is difficult to imagine the mosquito landscape in their absence. The native ranges of *Cx. pipiens* and *Cx. quinquefasciatus* are the Palearctic and African continent, respectively (Harbach 2012). The range expansion for these species likely coincided with human exploration and the development of trade routes. Given that *Cx. pipiens* originated from the northern hemisphere and *Cx. quinquefasciatus* the southern, it is not surprising that in North America *Cx. pipiens* has a northern range and *Cx. quinquefasciatus* a southern. However, there is a large zone of hybridization, covering most of California, where the ranges of the two species overlap (reviewed in Farajollahi et al. 2011). To further complicate the situation there are two forms of *Cx. pipiens,* form *pipiens* and form *molestus* Forskål. Form *molestus* is a less common variant, but has been detected in scattered North America areas, including the Sacramento Valley of California. Form *molestus* is a physiological variant of *Cx. pipiens* (Harbach et al. 1984), the key trait that separates it from both *Cx. quinquefasciatus* and form *pipiens* is autogeny, or the development of the first batch of eggs without first taking a bloodmeal.

In the Sacramento Valley, previous investigations into the *Cx. pipiens* complex demonstrated that collections of *Cx. pipiens* from the Sacramento County did not enter reproductive diapause, a physiological trait characteristic of *Cx. pipiens* but not *Cx. quinquefasciatus*. Additionally, autogenous populations were collected from multiple urban sources (Nelms et al. 2013). Genetic analysis of mosquitoes from the Sacramento Valley and the surrounding area revealed that mosquitoes that would have been identified by Vector Control Technicians as *Cx. pipiens* based on morphology and collection location were actually mixed assemblages of *Cx. quinquefasciatus*, *Cx. pipiens*, form *molestus*, and hybrids of the three (Kothera et al. 2013). These data confirmed that the Sacramento Valley is well within the *Cx. pipiens* / *Cx, quinquefasciatus* hybridization zone (Farajollahi et al. 2011) and that form *molestus* is established in the area (Kothera et al. 2013).

The taxonomic status of this species complex is a complicated puzzle. However, from a Mosquito Control District's perspective the most relevant question is whether these intermixed sub-populations, especially populations of form *molestus* that predominantly breed in urban sources, introduce new challenges for controlling the spread of vectorborne diseases. A previous study reported elevated insecticide resistance in form *molestus* collected by a nearby Northern California District (McAbee et al. 2004). Our goal for the present work, focusing on form *molestus*, was to assess the rates of autogeny in *Cx. pipiens* collected from Sacramento catch basins and to assess these sub-populations for insecticide resistance. Because autogenous egg development is a defining character of form *molestus*, this physiological trait was used to assess the extent that form *molestus* is established in the District's urban locations.

Methods

Two main study locations were identified based on the work of Nelms et al. (2013) and previous District observations of autogenous egg development. There were five collection locations in Land Park and six collection locations in East Sacramento. Grates on curbside catch basins were pulled back and adult mosquitoes were vacuumed from the basins. Immature mosquitoes were collected by dipper, then transferred to zip-top bags. Adult and immature mosquitoes were brought back to the laboratory for assessment.

Collected larvae and pupae were reared in mosquito 'breeder containers' (Bioquip; Rancho Dominguez, CA), and fed a protein-rich diet consisting of liver powder (Now Foods; Bloomingdale, IL) and inactivated yeast (Genesee Scientific; El Cajon, CA) in a ratio of 2:1. Adults were provided a 10% sucrose solution for subsistence, and females were dissected 5-10 days post-emergence to examine the ovaries for follicle development. Females with follicles containing mature eggs were clearly distinguishable from those with non-developing follicles. For colonization, adult females were briefly anesthetized with carbon dioxide and transferred to a chill table for inspection. Gravid and bloodfed females morphologically identified as *Cx. pipiens* were placed singly in narrow *Drosophila* vials (Flystuff; El Cajon, CA). Each tube contained a wooden probe, cut to fit completely in the tube, that served as a landing location. A single anesthetized female was added to each tube that was covered with tulle fabric secured by a rubber band. Once the mosquito roused, 10 ml of water infused with larval food was added to each tube to stimulate oviposition.

Egg rafts collected from tubes were reared singly in 8-cup (1890 ml) reusable food containers with lids. Containers were modified to add a screened ventilation hole to the lid and an aspiration port to the side of the container to allow for the collection of adults without unintentional release. Ports were covered with tape when not in use. Containers were filled with 1000ml of water. Egg rafts were reared as families and each container contained the egg raft from a single female. The developing larvae were fed a high protein food at a rate of 1.07 mg liver powder and 0.53 mg inactivated yeast per larvae per day. Pupae were picked from rearing containers and were allowed to emerge into a 1 gallon (3.78L) cardboard container. Adults were provided with 10% sucrose. Families that subsequently laid autogenous egg rafts were used to start a colony that was used for assessing insecticide susceptibility.

Bottle bioassay methods

Bottle bioassays were conducted to assess levels of insecticide susceptibility. Bottle bioassays methods were based on those previously compiled by the MVCAC Integrated Vector Management Committee (2016). Briefly, mosquitoes were tested against three different technical grade insecticides: deltamethrin (22mg/bottle), permethrin (43 mg/bottle), naled (10mg/bottle), and an acetone only control. Three technical replicates were prepared for each bottle and approximately 20 mosquitoes were added per bottle.

Four different populations were assessed for comparison: a susceptible colony of *Cx. quinquefasciatus* (CQ1), our newly formed *Cx. p. molestus* colony (SacMol), and two additional populations of *Cx. pipiens* (Frasinettis and Yorkshire) that were collected as gravid females from urban Sacramento County and the F1 progeny were reared for assessment without screening for autogeny. These populations were used to assess levels of resistance observed in local *Cx. pipiens* populations. The percentage of dead mosquitoes in test populations was compared to CQ1 after 120 min of exposure in the bottle. To compare the percentages of mortality for each pesticide assessed, a Kruskal-Wallis one-way analysis of variance on ranks was conducted followed by Tukey pairwise multiple comparisons with a 0.05 significance level.

Results and Discussion

Prevalence of Autogeny

Genetic analysis of *Cx. pipiens* populations was outside of the scope of this work. However, previous analyses (Kothera et al. 2013, Nelms et al. 2013) showed that mosquitoes from the study area that morphologically were identified as *Cx. pipiens*, but developed eggs autogenously, clustered together and were identified as form *molestus*. Prevalence of autogeny was calculated for six different catch basins from Downtown Sacramento and five different basins in the Sacramento neighborhood of Land Park (Table 1). The overall mean percentage of female mosquitoes from Downtown Sacramento (n=105) and Landpark (n=168) that developed eggs autogenously and were classified as form *molestus* was 38.7 (SD=15.2) and 19.2 (SD=11.6), respectively.

All sampling locations were curbside catch basins that during the mid-summer collection period predominately received water from landscape irrigation run off. Form *molestus* is well adapted for development in underground ecosystems. They are stenogamous (mating in tight spaces without a nuptial flight), tend to be mammalophilic (feed on mammals), and develop eggs autogenously, allowing them to complete the first gonotrophic cycle without taking a bloodmeal. In contrast *Cx. pipiens* is adapted for above ground ecosystems in that they are eurygamous (utilize a nuptial flight), are predominantly ornithophilic (feed on birds), and require a bloodmeal to complete the first gonotophic cycle (anautogenous) (Vinogradova 2003). Previous detections of form *molestus* in Boston (Spielman 1971), London (Byrne and Nichols 1998), Chicago (Mutebi and Savage 2009), and New York City (Kading 2012) were associated with purely underground habitats, with little access to above ground populations. In contrast the mosquitoes for our study were collected from gutter catch basins that were open, with only a grate blocking the top

Table 1.—Prevalence of autogenous egg development in urban populations of *Culex pipiens* across Sacramento, CA .

Basin ID	Study Area	Total Females	% Autogenous
FFl 8-303-08	Downtown Sacramento	13	53.8
FF 18-406-03	Downtown Sacramento	29	55.2
FF 18-804-01	Downtown Sacramento	18	22.2
FFl 8-804-08	Downtown Sacramento	18	44.4
FF18-888-14	Downtown Sacramento	5	20.0
FF18-888-15	Downtown Sacramento	22	36.4
JJl 3-306-01	Land Park	10	20.0
JJl 3-306-03	Land Park	13	7.7
JJ13-306-05	Land Park	52	11.5
JJl 3-606-07	Land Park	64	18.8
JJl 3-823-07	Land Park	29	37.9

entrance, and eventually drained into a combined sewer system. Depending on water levels, larval development could take place in the reservoir of water in the immediate basin or potentially deeper in the system.

These catch basins are productive mosquito sources and could more easily be classified as above rather than below ground sources. However, the detection of form *molestus* in these sites may indicate that they are established deeper in the sewer system and have spilled over into inlet basins, or that although they are highly adapted to underground ecosystems, they can also successfully utilize above ground sites [also see (Strickman and Fonseca 2012) who found autogeny in *Cx. pipiens* collected above ground]. Because both autogenous and anautogenous *Cx. pipiens* utilize these sources, there may be extensive opportunities for hybridization despite behavioral differences of the forms. It is still not clear whether the different detections of form *molestus* make up a monophyletic group or if the forms have developed repeatedly in an independent fashion. Large scale genetic analyses may shed light on this question.

Insecticide resistance

To compare levels of insecticide resistance the proportion of knocked down/dead mosquitoes, after 120 min of exposure in a bottle bioassay were assessed. The two field-collected *Cx. pipiens* populations (Frasinettis and Yorkshire) and the form *molestus* colony (SacMol) were compared to a susceptible laboratory population (CQ1).

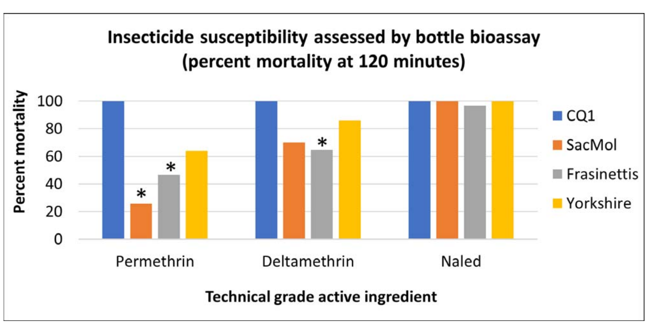

Figure 1.—Mean percent mortality of different *Culex pipiens* populations exposed to technical grade pesticide. Asterisks appear where test populations differed significantly from CQ1 (susceptible population).

Bottle bioassay results indicated that urban populations of *Cx. pipiens* have increased insecticide resistance levels to permethrin and to a lesser extent deltamethrin, compared to CQ1 (Fig. 1). Statistical analysis indicated that the SacMol colony had significantly lower mortality than CQ1 for Permethrin ($p = 0.05$), but no significant differences for deltamethrin or naled ($p > 0.05$).

Although the SacMol colony had elevated permethrin resistance compared to our susceptible colony, levels of resistance were similar to the other field-collected *Cx. pipiens* populations that served as a baseline for levels of insecticide resistance in the area. Although these populations were not assessed for autogeny, consistant levels of insecticide resistance between known autogenous and *Cx. pipiens* at large indicated that the insecticide exposure that is driving resistance is wide spread and likely affecting both autogenous and anautogenous populations. Form *molestus* is generally reported to be more mammaliophilic than the predominantly ornithophilic form *pipiens*. However, a limited bloodmeal identification study of genetically identified form *molestus* found that the majority of bloodmeals (10 out of 11) were from birds and that mammal feeding was less frequent (1 out of 11) (Nelms et al. 2013), perhaps indicating that these mosquitoes are opportunistic and will likely feed on both mammals and birds depending on host availability. Given their prevalence in urban environments, they may be important West Nile virus (WNV) vectors, bridging the virus to humans. However, because resistance levels in form *molestus* are similar to other urban breeding populations, control strategies that are effective against *Cx. pipiens* form *pipiens* populations will likely also be effective against form *molestus*. More study is needed to better understand the balance between form *molestus* and form *pipiens* in co-breeding situations, and the role form *molestus* plays as a WNV bridge vector.

Conclusions

Culex pipiens form *molestus* is well established in urban Sacramento. They were readily detected at two study sites in multiple catch basin sources. A colony of autogenous form *molestus* was established and assessed for insecticide resistance. Form *molestus* had significantly lower mortality than susceptible controls in bottle bioassays testing for permethrin. However, the form *molestus* colony was not significantly different from other urban populations of *Cx. pipiens* used as indicators of insecticide resistance in the area. Therefore, strategies that are effective against the current known levels of resistance levels will likely also be effective against form *molestus*.

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Surveillance for Mosquito-borne Encephalitis Virus Activity in California, 2019

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Abstract

In 2019, the California surveillance program for mosquito-borne encephalitis virus activity tested humans, horses, dead birds, mosquitoes, and sentinel chickens to detect arbovirus activity. West Nile virus (WNV) activity was reported from 35 out of 58 counties in California, and St. Louis encephalitis virus (SLEV) activity was reported from 12 counties. A total of 243 human WNV infections were reported, and enzootic WNV activity was detected among horses, dead birds, mosquitoes, and sentinel chickens. Six human cases of SLEV disease were identified in four counties, and enzootic SLEV activity was detected in mosquitoes and sentinel chickens.

INTRODUCTION

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

In 2019, the surveillance program included the following:

(1) Diagnostic testing of specimens from human patients who exhibited symptoms compatible with WNV disease as well as blood bank and organ donor screening for WNV infection.
(2) Monitoring mosquito abundance and testing mosquitoes for the presence of WNV, St. Louis encephalitis virus (SLEV), western equine encephalitis virus (WEEV), and other arboviruses as appropriate.
(3) Serological monitoring of sentinel chickens for WNV, SLEV, and WEEV antibodies.
(4) Reporting and testing of dead birds for WNV.
(5) Weekly reporting of arbovirus test results to ArboNET, the national arbovirus surveillance system.
(6) Weekly reporting of arbovirus activity in the CDPH Arbovirus Surveillance Bulletin and on the California WNV website: www.westnile.ca.gov.
(7) Data management and reporting of non-human data through the CalSurv Gateway, the California arbovirus surveillance system.

West Nile virus activity was reported from 35 (60%) out of 58 counties in California (Table 1), while SLEV activity was reported from 12 (21%) counties (Table 2).

HUMAN DISEASE SURVEILLANCE

Serological diagnosis of human infection with WNV and other arboviruses was performed at the CDPH Viral and Rickettsial Disease Laboratory (VRDL), seven county public health laboratories, and over 40 commercial laboratories. County laboratories tested for WNV using an IgM enzyme immunoassay (EIA) and/or an IgM immunofluorescence assay (IFA). Specimens with inconclusive results, or from counties with enzootic SLEV activity, were forwarded to the VRDL for further testing with a plaque reduction neutralization test (PRNT). Additional WNV infections were identified through screening performed by blood and organ donation centers.

In 2019, a total of 225 symptomatic and 18 asymptomatic infections with WNV were identified (Tables 1 and 3). Of the 225 symptomatic cases, 146 (65%) were classified as West Nile neuroinvasive disease (WNND) (e.g. encephalitis, meningitis, acute flaccid paralysis, or other neurologic dysfunction) and 79 (35%) were classified as non-neuroinvasive disease. Cases were residents of 27 (47%) counties and 136 (60%) were male. In 2019, WNV incidence in California was 0.56 cases per 100,000 persons. Incidence was highest (5.0 cases per 100,000 persons) in Fresno and Tulare counties; Fresno County also reported the most cases (51, 23% of total) (Figure 1, Table 3). The median age of those with WNND was 59.5 years (range, 10 to 93 years), and among cases with non-neuroinvasive disease the median age was 57 years (range, 19 to 86 years). The median age of

Table 1.—West Nile virus activity in California by county, 2019. Humans include asymptomatic infections detected through blood bank and organ donor screening. NT = None tested

County	Humans	Horses	Dead Birds	Mosquito Pools	Sentinel Chickens
Alameda	1	0	0	0	0
Alpine	0	0	NT	NT	NT
Amador	1	0	NT	NT	NT
Butte	6	0	1	44	34
Calaveras	0	0	NT	NT	0
Colusa	1	0	0	NT	6
Contra Costa	1	0	1	1	2
Del Norte	0	0	NT	NT	NT
El Dorado	1	0	0	NT	NT
Fresno	51	2	10	495	NT
Glenn	0	0	NT	1	1
Humboldt	0	0	0	NT	NT
Imperial	3	0	NT	2	NT
Inyo	0	0	NT	0	NT
Kern	32	2	2	129	NT
Kings	3	0	NT	63	NT
Lake	0	0	0	6	0
Lassen	0	0	NT	NT	NT
Los Angeles	31	0	53	94	28
Madera	4	2	NT	85	NT
Marin	0	0	0	0	NT
Mariposa	0	0	NT	NT	NT
Mendocino	0	0	NT	NT	NT
Merced	11	1	3	48	16
Modoc	0	0	NT	NT	NT
Mono	0	0	NT	0	NT
Monterey	0	0	0	NT	NT
Napa	0	0	0	0	NT
Nevada	0	0	0	NT	0
Orange	7	0	50	208	NT
Placer	1	0	3	53	8
Plumas	0	0	NT	NT	NT
Riverside	12	1	5	524	NT
Sacramento	4	1	44	74	4
San Benito	0	0	0	0	1
San Bernardino	11	1	4	52	NT
San Diego	3	0	2	0	NT
San Francisco	0	0	0	0	NT
San Joaquin	8	1	5	288	NT
San Luis Obispo	2	0	0	0	NT
San Mateo	0	0	0	0	0
Santa Barbara	0	0	0	0	0
Santa Clara	1	0	8	0	0
Santa Cruz	0	0	0	0	0
Shasta	0	0	1	3	1
Sierra	0	0	NT	NT	NT
Siskiyou	0	0	NT	NT	NT
Solano	1	0	0	5	4
Sonoma	0	0	0	0	NT
Stanislaus	17	2	4	203	NT
Sutter	2	0	0	15	11
Tehama	0	0	NT	NT	4
Trinity	0	0	NT	NT	NT
Tulare	25	0	17	813	10
Tuolumne	0	1	1	NT	NT
Ventura	2	1	2	1	3
Yolo	1	0	7	59	1
Yuba	0	0	3	22	5
State Totals	**243**	**15**	**226**	**3,288**	**139**

Table 2.—St. Louis encephalitis virus activity in California by county, 2019. NT = None tested

County	Humans	Mosquito Pools[1]	Sentinel Chickens
Fresno	2	58	NT
Imperial	2	5	NT
Kern	1	56	NT
Kings	0	4	NT
Los Angeles	0	2	0
Madera	0	5	NT
Merced	0	2	3
Orange	0	3	NT
Riverside	0	108	NT
San Bernardino	0	4	NT
Stanislaus	1	13	NT
Tulare	0	96	0
Totals	**6**	**356**	**3**

[1] Positive mosquito pools included *Cx. quinquefasciatus* (179 pools), *Cx. tarsalis* (171 pools), *Cx. pipiens* (5 pools), and *Cx. stigmatosoma* (1 pool)

the 6 WNV-associated fatalities was 77.5 years (range, 67 to 85 years). Dates of symptom onset ranged from May 12 to November 19, with the peak occurring in epidemiological week 36 (September 1- September 7), when 24 (11%) symptomatic infections were reported.

Six symptomatic cases of SLEV infection also were identified in 2019. All cases (100%) presented with neuroinvasive disease, and no fatalities were reported. Cases were residents of four counties (Table 2) and four (67%) were male. The median age was 64 years and dates of symptom onset ranged from July 14 to September 13.

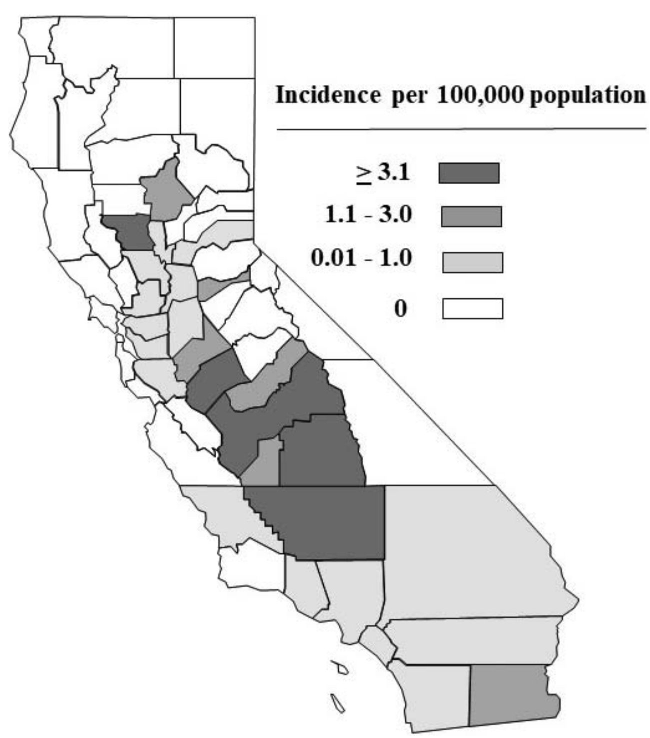

Figure 1.—Incidence of human cases of West Nile virus in California, 2019.

Table 3.—Reported West Nile virus human cases by county of residence and year, California, 2010 – 2019.

County	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2019 incidence per 100,000 person-years	Ten-year incidence per 100,000 person-years
Alameda	1	0	2	0	1	0	0	1	0	1	0.06	0.04
Alpine	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Amador	0	1	0	0	0	0	1	0	1	1	2.61	1.04
Butte	1	3	10	24	24	53	21	4	12	5	2.21	6.93
Calaveras	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Colusa	0	0	3	2	3	1	2	0	0	1	4.52	5.43
Contra Costa	4	3	4	5	5	1	4	4	4	1	0.09	0.30
Del Norte	0	0	0	0	0	0	0	0	0	0	0.00	0.00
El Dorado	0	1	0	1	0	0	1	0	0	0	0.00	0.16
Fresno	23	9	24	8	43	8	14	13	14	51	5.01	2.03
Glenn	2	1	7	9	10	19	6	0	2	0	0.00	19.22
Humboldt	0	0	0	0	0	0	0	0	1	0	0.00	0.07
Imperial	0	0	1	0	1	1	0	3	0	3	1.58	0.47
Inyo	0	0	0	0	0	0	0	4	0	0	0.00	2.15
Kern	15	18	25	25	11	11	17	30	13	28	3.06	2.11
Kings	1	1	3	1	4	0	8	5	0	3	1.95	1.69
Lake	0	0	1	0	1	2	1	0	1	0	0.00	0.92
Lassen	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Los Angeles	4	58	163	151	253	286	151	277	43	31	0.30	1.38
Madera	7	2	3	3	3	4	6	2	4	3	1.88	2.32
Marin	0	0	0	2	0	1	0	0	0	0	0.00	0.11
Mariposa	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Mendocino	0	0	0	0	1	2	0	0	0	0	0.00	0.34
Merced	1	1	13	0	1	1	0	10	2	10	3.53	1.38
Modoc	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Mono	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Monterey	0	0	1	0	0	0	1	0	1	0	0.00	0.07
Napa	0	0	0	1	0	0	0	0	1	0	0.00	0.14
Nevada	0	0	0	0	0	2	0	0	1	0	0.00	0.30
Orange	1	10	42	10	263	92	32	33	9	5	0.16	1.54
Placer	3	1	12	6	7	0	7	0	9	1	0.25	1.16
Plumas	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Riverside	0	7	19	35	14	127	11	32	15	12	0.49	1.11
Sacramento	12	4	29	11	10	4	25	6	15	4	0.26	0.78
San Benito	0	0	0	0	0	0	0	0	0	0	0.00	0.00
San Bernardino	5	4	33	13	21	54	8	57	9	7	0.32	0.96
San Diego	0	0	1	0	11	42	20	2	2	3	0.09	0.24
San Francisco	1	0	1	1	0	0	0	1	0	0	0.00	0.05
San Joaquin	6	5	13	8	9	2	13	14	14	7	0.91	1.18
San Luis Obispo	0	0	0	0	0	0	0	0	0	2	0.71	0.07
San Mateo	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Santa Barbara	0	1	0	1	0	0	0	0	0	0	0.00	0.04
Santa Clara	0	1	0	2	10	8	1	0	1	1	0.05	0.12
Santa Cruz	0	1	0	0	0	0	0	0	0	0	0.00	0.04
Shasta	0	0	1	1	2	3	1	1	1	0	0.00	0.56
Sierra	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Siskiyou	0	0	0	0	0	1	0	0	0	0	0.00	0.22
Solano	0	0	2	1	5	1	4	1	0	1	0.23	0.34
Sonoma	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Stanislaus	12	11	26	17	33	13	26	28	15	16	2.86	3.52
Sutter	0	0	8	10	8	2	12	3	1	1	1.03	4.62
Tehama	0	1	4	5	4	5	5	2	2	0	0.00	4.35
Trinity	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Tulare	12	11	7	5	21	13	10	12	8	24	5.01	2.57
Tuolumne	0	0	0	0	0	0	0	0	1	0	0.00	0.18
Ventura	0	0	7	2	1	6	7	1	2	2	0.23	0.33
Yolo	0	0	10	6	15	8	16	6	11	1	0.45	3.28
Yuba	0	3	4	13	6	10	11	1	2	0	0.00	6.42
Total WNV Cases	**111**	**158**	**479**	**379**	**801**	**783**	**442**	**553**	**217**	**225**	**0.56**	**1.04**
Asymptomatic Infections	20	18	48	54	91	77	41	47	26	18		
Total WNV infections	**131**	**176**	**527**	**433**	**892**	**860**	**483**	**600**	**243**	**243**	**0.61**	**1.15**

Table 4.—Results of mosquito and sentinel chicken testing for West Nile virus, California, 2019.

County	No. mosquitoes tested	No. mosquito pools tested	WNV + pools	No. flocks	No. chickens	No. WNV positive flocks	WNV + sera
Alameda	18,086	1,438	0	2	12	0	0
Butte	19,350	400	44	7	45	7	34
Calaveras	0			1	10	0	0
Colusa	0			1	10	1	6
Contra Costa	15,468	456	1	5	50	1	2
Fresno	63,386	2,001	495	0			
Glenn	1,248	27	1	1	6	1	1
Imperial	1,016	33	2	0			
Inyo	1,618	38	0	0			
Kern	37,095	878	129	0			
Kings	16,725	518	63	0			
Lake	18,651	715	6	2	11	0	0
Los Angeles	162,733	4,586	94	28	190	10	28
Madera	13,620	344	85	0			
Marin	3,100	184	0	0			
Merced	19,832	840	48	8	48	6	16
Mono	50	1	0	0			
Napa	4,793	139	0	0			
Nevada	0			2	12	0	0
Orange	126,251	5,512	208	0			
Placer	47,019	2,785	53	3	18	2	8
Riverside	253,055	7,027	524	0			
Sacramento	61,215	4,578	74	3	19	1	4
San Benito	300	21	0	1	10	1	1
San Bernardino	80,642	3,744	52	0			
San Diego	36,556	2,592	0	0			
San Francisco	102	10	0	0			
San Joaquin	85,500	2,667	288	0			
San Luis Obispo	2,263	62	0	0			
San Mateo	3,852	118	0	2	14	0	0
Santa Barbara	8,094	182	0	5	34	0	0
Santa Clara	1,579	134	0	8	56	0	0
Santa Cruz	3,682	263	0	2	20	0	0
Shasta	11,192	442	3	7	50	1	1
Solano	19,684	833	5	3	36	2	4
Sonoma	15,422	586	0	0			
Stanislaus	62,378	1,685	203	0			
Sutter	8,281	252	15	5	35	3	11
Tehama	0			3	29	2	4
Tulare	116,628	3,915	813	1	11	1	10
Ventura	2,757	62	1	5	54	2	3
Yolo	44,762	1,970	59	2	10	1	1
Yuba	6,405	195	22	1	7	1	5
Total	**1,394,390**	**52,233**	**3,288**	**108**	**797**	**43**	**139**

MOSQUITO SURVEILLANCE

In 2019, mosquito testing was performed at DART and 12 local mosquito and vector control agencies. A total of 1,394,390 mosquitoes (52,233 pools) collected in 39 counties were tested by a real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) for SLEV, WEEV, and/or WNV viral RNA (Table 4). *Aedes aegypti* and *Ae. albopictus* mosquitoes also were tested for chikungunya, dengue, and Zika viruses at DART by a separate RT-qPCR.

West Nile virus was detected in 3,288 mosquito pools from 25 counties (Tables 1 and 4), and SLEV was detected in 356 mosquito pools from 12 counties (Table 2). Statewide, the annual minimum infection rate (MIR-defined as the minimum number of infected female mosquitoes per 1,000 tested) of WNV in all mosquitoes tested was 2.4. During California's peak transmission period (July – September) the statewide MIR in *Culex* mosquitoes was 3.9 and five counties reported MIRs greater than 5.0, the epidemic threshold value (Figures 2 and 5) (California Department of Public Health, 2019).

West Nile virus was identified from five different *Culex* species (*Cx. erythrothorax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, and *Cx. tarsalis), Aedes aegypti, and Culiseta incidens* (Table 5); positive pools were collected from January 29 – November 19, with the peak occurring in epidemiological week 33 (August 11 – August 17). St. Louis encephalitis virus also was identified from four *Culex* species (*Cx. pipiens*, *Cx. quinquefasciatus*, *Cx.*

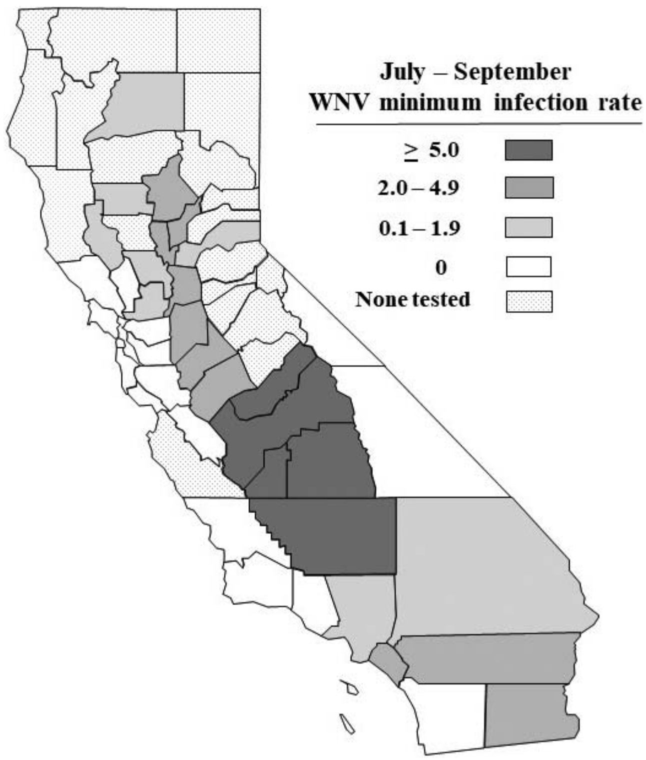

Figure 2.—West Nile virus minimum infection rate in *Culex* mosquitoes, by county, California, July – September, 2019. Minimum infection rate is defined as the minimum number of infected female mosquitoes per 1,000 tested.

stigmatosoma, and *Cx. tarsalis)* collected from May 24 – November 13.

A total of 15,204 *Ae. aegypti* and 154 *Ae albopictus* were tested for chikungunya, dengue, and Zika viruses; all were negative.

CHICKEN SEROSURVEILLANCE

In 2019, 27 local mosquito and vector control agencies in 25 counties maintained 108 sentinel chicken flocks (Table 4). Blood samples were collected from chickens every other week and tested for IgG antibodies to WNV, SLEV and WEEV by an EIA at the CDPH Vector-Borne Disease Section Laboratory and one local agency. Presumptive positive samples were confirmed by IFA or western blot. Samples with inconclusive results were tested by PRNT at the VRDL.

Of 9,160 chicken blood samples tested, 139 seroconversions to WNV were detected among 43 (40%) flocks in 17 counties (Tables 1 and 4). Seroconversions to WNV occurred between July 25 and November 14, with the peak occurring during epidemiological weeks 34-35 (August 18 – August 31). In addition, three SLEV seroconversions were detected in two flocks located in Merced County between August 30 and September 13 (Table 2).

DEAD BIRD SURVEILLANCE

In 2019, the WNV Dead Bird Call Center and website received a total of 5,681 dead bird reports from the public from 53 counties (Table 6). Oral swabs or tissue samples from dead bird carcasses were tested at DART or at one of 12 local agencies by RT-qPCR. Of the 1,927 bird carcasses that were deemed suitable for testing, WNV was detected in 226 (12%) carcasses from 21 counties (Tables 1 and 6). Twenty-two different bird species tested positive for WNV: 62% were American crows, 16% were California scrub-jays, 3% were other corvids, and 19% were non-corvid species. Positive birds were detected from March 12 – December 4, with the peak occurring in epidemiological week 38 (September 15 – September 21).

HORSES

Serum or brain tissue specimens from horses displaying neurological symptoms were tested for WNV at the California Animal Health and Food Safety Laboratory. In 2019, WNV infection was confirmed in 15 horses from 12 counties (Table 1). Three (20%) of the horses died or were euthanized as a result of their infection.

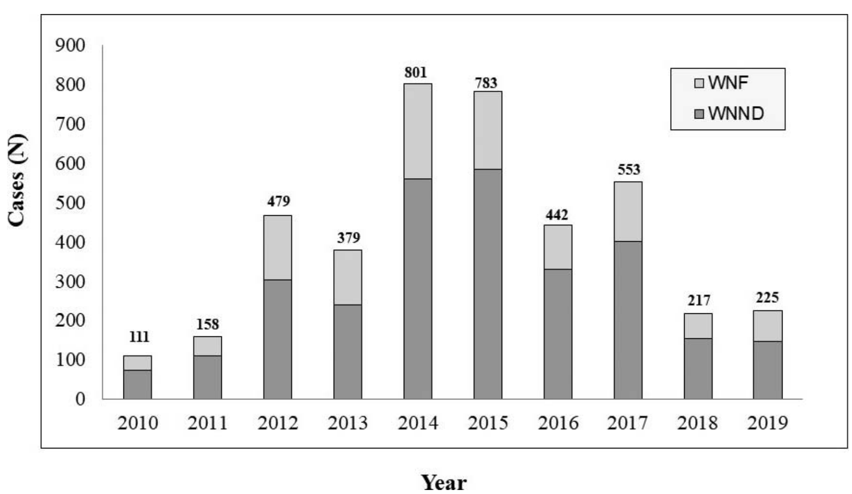

Figure 3.—Human cases of West Nile virus in California, by year, 2010 – 2019.

Table 5.—Mosquito species tested for West Nile virus, California, 2019.

Culex species	No. Pools	No. mosquitoes	WNV +	MIR
Cx. erythrothorax	2,900	95,552	2	0.02
Cx. pipiens	8,937	171,772	373	2.2
Cx. quinquefasciatus	20,092	582,174	1,954	3.4
Cx. restuans	2	3	0	0.0
Cx. stigmatosoma	1,317	15,461	28	1.8
Cx. tarsalis	17,387	512,586	929	1.8
Cx. thriambus	179	519	0	0.0
Culex species	33	127	0	0.0
All *Culex*	**50,847**	**1,378,194**	**3,286**	**2.4**
Anopheles species	Pools	No. mosquitoes	WNV +	MIR
An. franciscanus	12	89	0	0.0
An. freeborni	8	72	0	0.0
An. hermsi	15	236	0	0.0
All *Anopheles*	**35**	**397**	**0**	**0.0**
Aedes species	Pools	No. mosquitoes	WNV +	MIR
Ae. aegypti	767	6,540	1	0.2
Ae. albopictus	5	74	0	0.0
Ae. nigromaculis	2	43	0	0.0
Ae. notoscriptus	2	4	0	0.0
Ae. sierrensis	1	3	0	0.0
Ae. squamiger	1	19	0	0.0
Ae. taeniorhynchus	7	303	0	0.0
Ae. vexans	3	94	0	0.0
Ae. washinoi	2	27	0	0.0
All *Aedes*	**790**	**7,107**	**1**	**0.1**
Other species	Pools	No. mosquitoes	WNV +	MIR
Culiseta incidens	448	6,852	1	0.1
Culiseta inornata	78	408	0	0.0
Culiseta particeps	13	381	0	0.0
Unknown	22	1,051	0	0.0
All other	**561**	**8,692**	**1**	**0.1**

DISCUSSION

In 2019, 225 WNV human cases were reported from 27 counties, which was the second lowest number of cases reported in California since 2011 (Figure 3, Table 3). However, the proportion of WNND cases among reported cases in California was 65%, which suggests that up to 10,000 non-neuroinvasive cases also occurred, but were not identified or reported, as these infections are less likely to be identified, laboratory tested, and reported (Figure 3) (Centers for Disease Control and Prevention, 2010). Non-human WNV activity was reported from 31

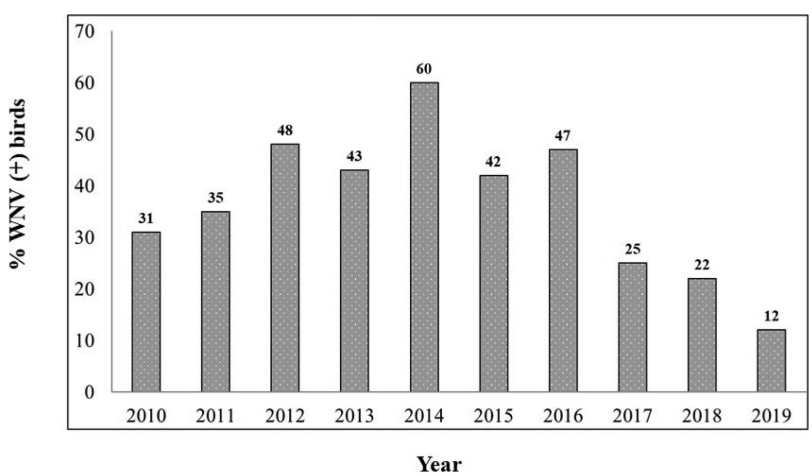

Figure 4.—Percentage of dead birds positive for West Nile virus in California, 2010 – 2019.

Table 6.—Dead birds reported, tested, and positive for West Nile virus, California, 2019.

County	Reported	Tested	Positive	(%)
Alameda	213	83	0	(0)
Alpine	0			
Amador	4	0		
Butte	67	21	1	(4.8)
Calaveras	8	0		
Colusa	7	1	0	(0)
Contra Costa	321	23	1	(4.3)
Del Norte	0			
El Dorado	47	14	0	(0)
Fresno	344	16	10	(62.5)
Glenn	3	0		
Humboldt	8	4	0	(0)
Imperial	1	0		
Inyo	14	0		
Kern	27	2	2	(100)
Kings	15	0		
Lake	12	2	0	(0)
Lassen	2	0		
Los Angeles	694	182	53	(29.1)
Madera	9	0		
Marin	53	5	0	(0)
Mariposa	1	0		
Mendocino	7	0		
Merced	40	5	3	(60.0)
Modoc	0			
Mono	1	0		
Monterey	21	5	0	(0)
Napa	25	1	0	(0)
Nevada	4	3	0	(0)
Orange	621	489	50	(10.2)
Placer	184	119	3	(2.5)
Plumas	2	0		
Riverside	116	40	5	(12.5)
Sacramento	738	317	44	(13.9)
San Benito	3	1	0	(0)
San Bernardino	105	20	4	(20.0)
San Diego	175	124	2	(1.6)
San Francisco	47	5	0	(0)
San Joaquin	137	33	5	(15.2)
San Luis Obispo	29	6	0	(0)
San Mateo	233	61	0	(0)
Santa Barbara	22	2	0	(0)
Santa Clara	442	151	8	(5.3)
Santa Cruz	94	22	0	(0)
Shasta	19	1	1	(100)
Sierra	0			
Siskiyou	1	0		
Solano	55	11	0	(0)
Sonoma	54	6	0	(0)
Stanislaus	142	15	4	(26.7)
Sutter	49	10	0	(0)
Tehama	6	0		
Trinity	0			
Tulare	135	31	17	(54.8)
Tuolumne	15	2	1	(50.0)
Ventura	92	18	2	(11.1)
Yolo	181	67	7	(10.4)
Yuba	36	9	3	(33.3)
Totals	**5,681**	**1,927**	**226**	**(11.7)**

counties, but virus activity was low in most areas of the state, with the exception of the Coachella Valley (Riverside County) and the South San Joaquin Valley regions. Notably, Fresno and Tulare counties each reported more human cases in 2019 than in any other year since 2005 (Table 3; California Department of Public Health). Correspondingly, the mosquito MIR during July - September was also highest in Fresno County (10.5), followed by Tulare County (9.9) (Figure 2). Statewide, WNV activity in mosquitoes and sentinel chickens was amongst the lowest detected since 2010, and the number and percent of WNV positive dead birds (226, 12%) were the lowest reported since 2003 (Figures 4, 5, and 6; California Department of Public Health). Similar to previous years, ecological surveillance data documented WNV activity throughout the year, but the vast majority of detections occurred from June through October, with peak activity occurring in August.

For the fifth consecutive year, SLEV was also detected in California. Outreach to local health departments was conducted in areas after enzootic detections of SLEV and medical providers were encouraged to include SLEV testing for suspect WNV cases. This resulted in the identification of six human SLEV disease cases from four counties, the highest number reported in California since SLEV reemerged in 2015. Enzootic activity was detected in 12 counties via mosquito and sentinel chicken testing, but most detections were reported from Fresno, Kern, Riverside, and Tulare counties, where elevated WNV activity also was reported (Table 2). Only three sentinel chickens from one county (Merced) tested positive for SLEV infection, but sentinel flocks were absent from almost all counties where SLEV was detected in mosquitoes (Table 2). Of note, the first indicator of SLEV activity in Merced County was a SLEV seropositive chicken, highlighting the importance of utilizing multiple surveillance methods to increase sensitivity of the surveillance system.

Although WEEV has not been detected in California since 2007, routine testing of mosquitoes and sentinel chickens for WEEV has continued in the event this historically endemic arbovirus reemerges.

CONCLUSIONS

Statewide, WNV activity was low in 2019 and only 225 human WNV cases were reported. However, significantly elevated virus activity still occurred in some counties, highlighting the importance for ongoing surveillance and awareness of potential human disease risk. Six human cases of SLEV disease were identified in 2019, along with enzootic detections in 12 counties. In most areas, environmental detections of both viruses preceded the incidence of human cases, emphasizing the value of environmental surveillance to direct mosquito control efforts and decrease the risk arboviral diseases in California.

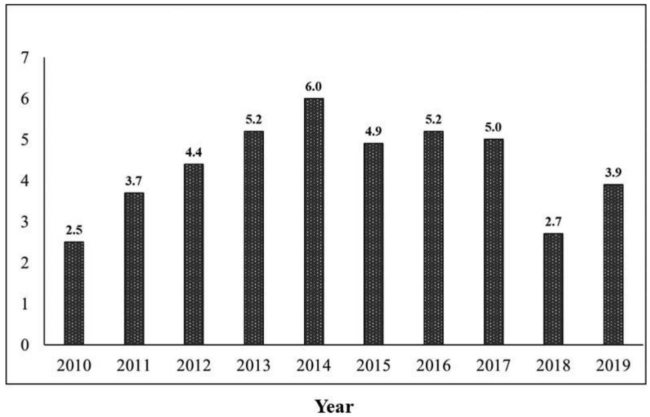

Figure 5.—Minimum infection rate (MIR) in females per 1,000 tested for West Nile virus in *Culex* mosquitoes in California, July – September, 2010–2019.

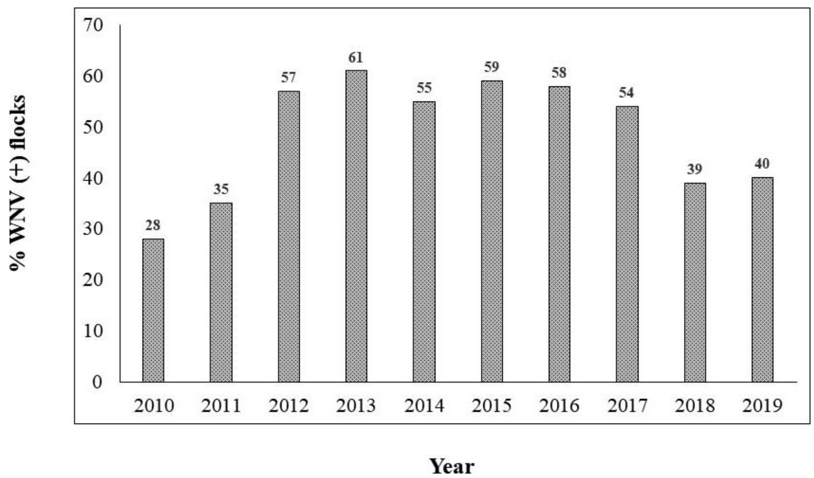

Figure 6.—Percentage of sentinel chicken flocks in California with one or more birds positive for antibodies to West Nile virus, 2010–2019.

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Aedes mosquitoes and Zika, chikungunya, and dengue in California: An update

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Abstract

This presentation summarized the distribution of the invasive mosquito species, *Aedes aegypti* and *Aedes albopictus*, in California through 2019. In particular, *Ae. aegypti* expanded its range in 2019 to four new counties in northern California: Stanislaus, San Joaquin, Sacramento, and Placer. *Aedes aegypti* is the primary vector of Zika, chikungunya, and dengue viruses, and *Ae. albopictus* is a secondary vector. Although local transmission of these viruses has not been detected in California, travel-associated cases have been reported. Information was provided on the number of cases reported in California since 2015 by county, annual trends, and travel history. Resources pertaining to *Aedes* and *Aedes*-borne diseases available on the California Department of Public Health website were summarized (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/Aedes-aegypti-and-Aedes-albopictus-mosquitoes.aspx).

Where has all the West Nile virus gone?

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Introduction

West Nile virus (WNV) activity in Contra Costa County in 2019 was the lowest since the virus was first detected here in 2005. Given the low levels of virus detected and a decline in dead bird reports, we decided to re-evaluate the effectiveness of the individual components of our surveillance program for predicting human case risk.

Methods

Data were collected during normal mosquito and arbovirus surveillance activities from 2005 through 2019. Approximately 22-23 New Jersey light traps and 35-45 dry-ice baited EVS traps were operated weekly at fixed and 'random' surveillance sites. Pools of 10-50 mosquitoes from EVS traps were submitted to the DART laboratory at UC Davis for virus testing, dead corvids reported to the West Nile virus hotline were collected and submitted for testing, and five flocks of sentinel chickens were monitored for antibodies to WNV and other arboviruses. Human case data were provided by the Contra Costa County Department of Public Health.

Results and Discussion

During 2019 WNV was first detected in a sentinel chicken near Knightsen on August 26th and in a dead crow collected in Danville on September 3rd , which was our latest detection since the virus first appeared in our County in 2005. Dead bird reports were low, continuing a steady decline which appears to be due to a decrease in public interest in the West Nile hotline, rather than the development of herd immunity in the wild bird population, since infection rates in birds have not shown a similar decline. Historically WNV positive dead birds have been our best early season indicator of virus activity and have showed the strongest correlation with human cases. However, for the past two seasons, seropositive sentinel chickens have provided the earliest indication of WNV transmission, and showed the second-strongest correlation with human cases after WNV positive birds. Numbers of positive mosquito pools did not appear to be predictive of human cases.

Conclusions

With the low levels of WNV transmission observed in the past two years, and an apparent decline in public reporting of dead birds, we may need to re-evaluate our surveillance strategies. Sentinel chickens, once widely used because they were a sensitive indicator for WEE and SLE viruses, have been reduced or eliminated by some surveillance programs statewide, because they appeared to be a less sensitive indicator for WNV transmission than dead birds. However, if dead bird reports continue to decline, sentinel chickens may provide the next-best mechanism for detection of human case risk.

Acknowledgments

The authors thank our seasonal laboratory aides, Jason Hernandez and David Hojas, as well as the entire Operations crew, Program Supervisors, and General Manager Paula Macedo for their assistance and support throughout the season.

Regional variation in California's West Nile virus dead bird surveillance program

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Abstract

The West Nile virus (WNV) dead bird surveillance program (DBSP) provided early and ongoing WNV detection data to help guide mosquito control efforts and public messaging in 2019. Year-end data for the DBSP was summarized, with attention to dead bird report volume and species reported. Nearly half of all dead bird reports were received via live calls, and call volume in 2019 was lower than the 5-year average. Low call volume corresponded with low overall WNV activity (12% prevalence) in tested dead birds, and a downward trend in human cases in recent years. The importance of corvids in WNV surveillance and ecology was discussed.

Impacts of an unprecedented year for virus activity in the Coachella Valley

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INTRODUCTION

West Nile Virus (WNV) dispersal patterns in the Coachella Valley region of Southern California have been well documented (Lothrop et al. 2008). Typically dispersal starts from activity in rural areas around the Salton Sea north shoreline where the main vector is *Culex tarsalis*. Then as the season progresses, the virus disperses northward towards the urban areas where *Cx. quinquefasciatus* is the main vector. However, since the introduction of St. Louis encephalitis virus (SLEV), there has been an early season detection of WNV in the urban area for a short time, followed by mainly rural SLEV and WNV detections along the shoreline of the Salton Sea, and then late season activity again progressing northward towards the urban areas. Conversely, in 2019, virus initially was detected at multiple urban cities and then concurrently in both urban and rural areas (Fig. 1). Detections remained in the urban area for a longer duration and were more widespread than in previous years. Overall, 2019 was a record year, with over 600 positive pools detected among >200,000 mosquitoes tested, culminating in a record prevalence of 9.8% positive pools, as compared to 1.7-3.9% positive samples from the previous 4 years (Table 1).

Interestingly, during recent years, SLEV and WNV activity seems to alternate from year to year, where the number of positive WNV samples are greater in odd years and SLEV positive samples are greater in even years. The peak WNV and SLEV infection rates appear to be temporally distinct (Fig. 2), with WNV occurring earlier in the season with a peak in July (Wk 30) and SLEV occurring later with a peak in September (Wks 36 - 38).

METHODS

Coachella Valley Mosquito and Vector Control District's (the District) virus response follows the guidelines summarized in the California Mosquito-Borne Virus Surveillance and Response Plan published by the California Department of Public Health (2019). Once virus detection is confirmed, the virus risk is assessed and treatment response determined. Typically adulticides are used for the District's virus response. With that, the District considers the limitations of adulticides based on the label restrictions and the level of insecticide resistance to the products. The District also has regions in the Coachella Valley that harbor protected species that may be impacted by the application (USFWS). Normally, in urban areas, trucks are used for applications and in rural areas, aerial treatments can cover locations that a truck cannot reach. After determining the product, application method, and treatment dates and time, the District notifies the residents and stakeholders.

RESULTS AND DISCUSSION

In 2019, the District completed more nights of truck ultra-low volume (ULV) treatments and aerial adulticide applications than in any previous year. The District conducted 131 ULV truck treatments, 43 aerial adulticide applications in the rural area, and 3 aerial adulticide applications in Indio. A total of 1,560 hours of overtime or compensated time were accrued by the staff. This doesn't account for the staff that worked a modified schedule in lieu of overtime.

New District response strategies were conducted to address the virus activity in 2019. Aerial larvicide applications were conducted in Palm Springs for the first time to address these viruses, in addition to reducing the *Aedes aegypti* abundance. Using larvicides in conjunction with adulticides in Palm Springs was an effort to reduce adult numbers for a longer time period. Additionally, the District conducted aerial adulticide applications over the city of Indio. The aerial treatments were done here to relieve the work load from our technicians and cover an area larger than a truck can cover in one night. By late June, WNV had been detected repeatedly in Indio, despite conducting larvicide and truck ULV mosquito control applications. After the aerial application, virus was not detected in mosquitoes for 4 wks post-treatment and fewer adult mosquitoes were collected per trap night. With this success, we plan to retain aerial adulticiding as a control option for residential areas.

The greater number of aerial and ground applications conducted for virus response meant that operating expenses were higher than expected for the 2019-2020 fiscal year (Table 2). Having a contingency budget for these unexpected expenses helped buffer these costs.

During 2019 we collected 3-5 times more mosquitoes per trap in spring through early-summer in the rural and urban areas when compared to the 5 year average (Fig. 3), which

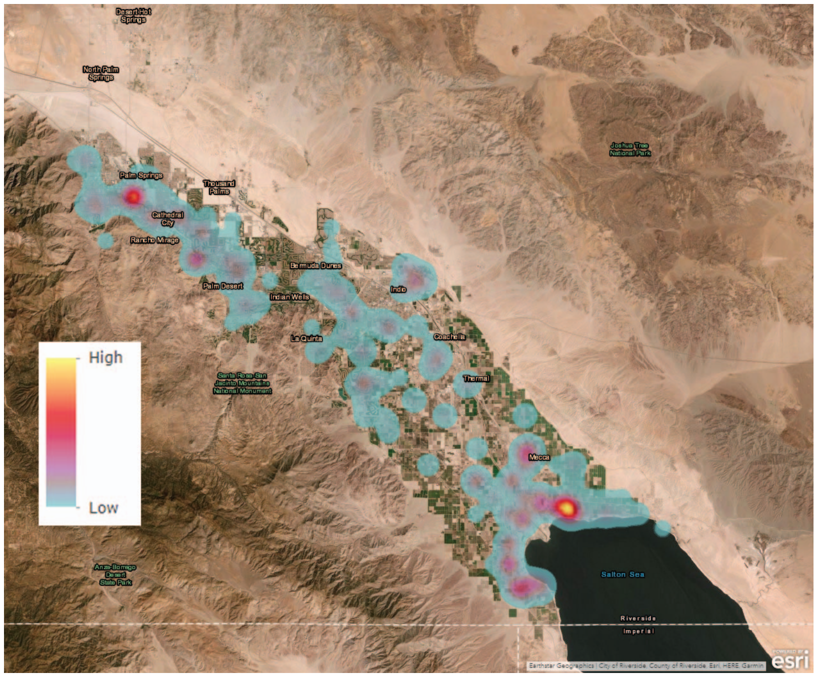

Figure 1.—Heat map showing the number of positive virus pools detected in 2019.

Table 1.—Female *Culex* mosquito samples tested and virus detections for 2015-2019.

	2015	2016	2017	2018	2019
Pools Tested	3,903	4,523	5,148	4,337	6,168
Number of Mosquitoes	112,248	66,893	154,510	140,529	218,342
Pools WNV Positive	99	19	120	24	513
Pools SLEV Positive	37	92	23	56	105
% Positive (both viruses)	3.4%	3.9%	2.7%	1.7%	9.8%

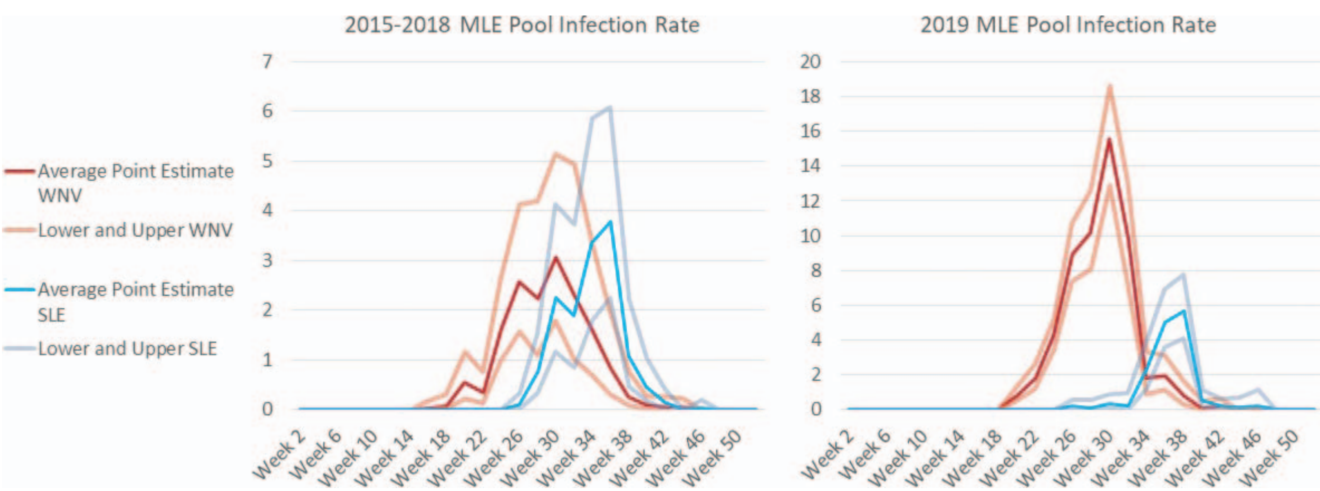

Figure 2.—Bias-corrected maximum likelihood estimate (MLE) of the WNV and SLEV infection rate per 1,000 *Culex* females tested, with 95% confidence intervals, for District-wide biweekly pools from 2015-2018 (left) and 2019 (right). Values were calculated using the CalSurv pool infection rate calculator.

Table 2.—Fiscal year 2019 (7/1/19-11/30/19) budget expenses in dollars directly impacted by control operations. The amount allotted for the fiscal year is the annual budget. The YTD budget is the expected amount spent for the time period and YTD actual is the actual expenses for the time period. YTD budget variance is the difference between these two with over expenditures in bold.

	Annual Budget	YTD Budget	YTD Actual	YTD Budget Variance
Payroll - Overtime Expense	18,700	7,792	17,678	**(9,886)**
Control Products	785,000	327,083	627,941	**(300,858)**
Aerial Applications	124,500	51,875	131,875	**(80,000)**
Contingency Expense	150,000	62,500	34,398	28,102

may have contributed to the high virus levels during the season. An early-season action threshold without virus detections is being considered to reduce mosquito abundance and perhaps prevent elevated virus levels later in the season.

Because of the high infection rate and mosquito abundance, the District remained in emergency planning risk level in regards to the state risk assessment model for WNV (CDPH 2019). This may have been due, in part, to the low value for dead birds, which brought down the overall risk level. The District does not receive many dead bird calls nor do we test many birds. Since 2015, we have tested an average of 3.4 birds per season which were all negative for virus. The 4 birds tested this year also were negative for virus. The last time a bird tested positive for virus was in 2013. The District is reevaluating components for the risk assessment model.

Previous work (Diaz et al. 2018; White et al. 2016) indicated that SLEV detections in the Coachella Valley may be linked with detections in Arizona. District staff monitored the virus activity in Arizona through publicly available webpages, later including activity in Nevada as the season progressed. One author (Henke) participated in Arizona monthly phone calls about mosquitoes and mosquito-borne diseases where all agencies shared their surveillance results and human case numbers. This partnership outside of California but within neighboring jurisdictions reinforced that the virus activity the District observed was not in isolation.

CONCLUSION

The 2019 season was a test of the District's ability to address an extended period of high levels of virus over a large area. Ultimately, the District finished the trying season with 9 reported human cases, which was 3 more cases than the last high-WNV season in 2017. This experience will guide the District towards improving virus response capabilities in the coming years. One of the efforts to improve virus response is to review and implement a Mutual Aide Agreement among the vector control agencies of the MVCAC Southern Region. This agreement will allow sharing of resources such as personnel and equipment among agencies when there is a need. These resources can be deployed immediately to help other districts when a timely response is essential for disrupting virus transmission and protecting public health.

ACKNOWLEDGEMENTS

The work described herein reflects the dedicated performance of all the certified vector control technicians

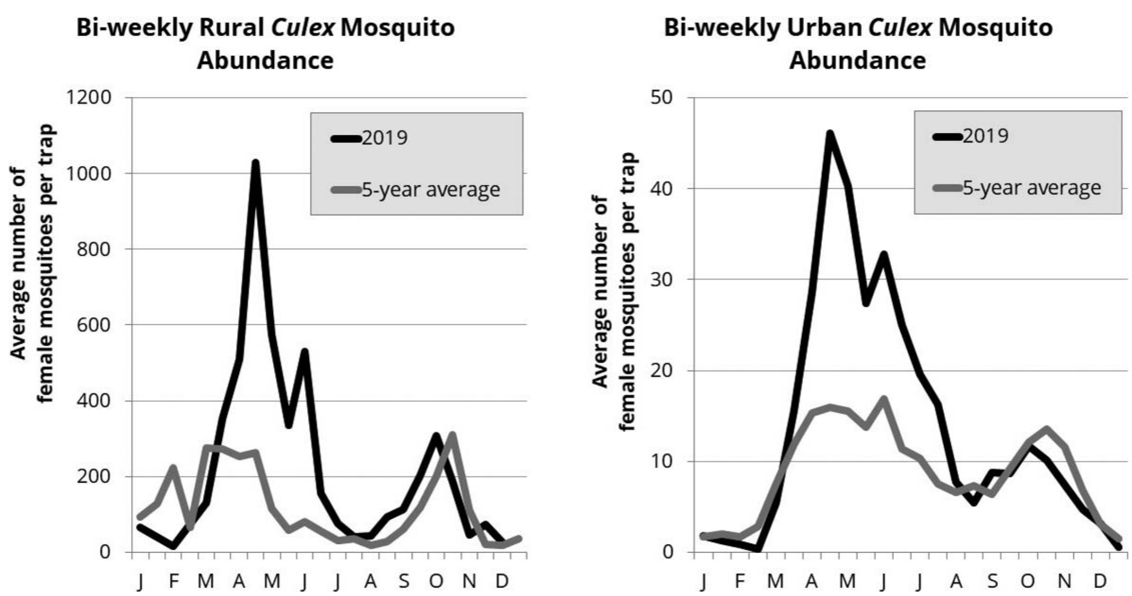

Figure 3.—Biweekly *Cx. tarsalis* and *Cx. quinquefasciatus* mosquito abundance from CO_2 and gravid traps in the rural area (left) and the urban area (right) in 2019 compared to the 5-year average. Values were calculated using the CalSurv abundance anomaly model.

who completed the many early morning treatments, which includes staff from the facilities, finance, fleet, information technology, laboratory, operations, and outreach departments. Thanks to David I'Anson, the District's Administrative Finance Manager, for reviewing the District expenses. We thank San Gabriel Valley Mosquito and Vector Control District for loaning us several pieces of equipment to help conduct our work. We also thank the Salton Sea Air Service and Hummingbirds, Inc. for conducting the many aerial treatments for the 2019 season.

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Significance of Underground Storm Drain Systems (USDS) Breeding Sites for Invasive *Aedes aegypti* and *Aedes albopictus* Mosquitoes in Southern California

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INTRODUCTION

Underground storm drain systems (USDS) in southern California are comprised of street-level catch basins and a variety of subsurface stormwater infrastructures that transport water from streets to large open flood control channels, water impoundment basins, or directly into the Pacific Ocean. These systems are widely distributed in urban areas and are a significant source of *Culex quinquefasciatus* Say (Kluh et al. 2001, Su et al. 2003, Cummings et al. 2019). Whether the USDS habitat is attractive to ovipositon and is suitable for exploitation by container-breeding, invasive *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse) in southern California is uncertain. Therefore, the objective of the current study was to examine the oviposition behavior, egg hatching rates, and larval survival of immature *Ae. aegypti* and *Ae. albopictus* in water collected from underground storm drains. This information can be used to infer the potential utilization of the USDS by invasive *Aedes* in highly urbanized areas of California.

METHODS

Oviposition preference

To examine whether USDS water was attractive or repellent to oviposition by invasive *Aedes* mosquitoes, a two-choice oviposition preference test was conducted. This experiment used two ovicups placed within a mosquito cage ($1 \times 0.5 \times 0.5$ m^3), one with 200 ml of water from USDS sites and another with 200 ml of water collected from flowerpots. Ten gravid *Ae. aegypti* or *Ae. albopictus* females were released into species-specific cages and allowed to lay eggs for 3 days. The number of eggs in each ovicup was counted. Five replicates were used for each water type per species, for a total of 50 gravid females/species/water type.

Egg hatching

To investigate the effects of USDS water on egg hatching, 50 *Ae. aegypti* or *Ae. albopictus* eggs were introduced into ovicups with USDS or field-collected flowerpot water, and the number of larvae hatched were counted daily for 6 days. Five replicates were used for each type of larval habitat water per species, for a total of 250 eggs/species/water type.

Larval survivorship

To determine the impact of USDS water on larval survivorship, 25 newly hatched *Ae. aegypti* or *Ae. albopictus* larvae were introduced into separate trays with USDS or field collected flowerpot water. The number of surviving larvae were counted until they pupated. Four replicates were used for each type of larval habitat water per species, for a total of 100 larvae/species/water type.

This experiment was conducted during September-October 2019 when invasive *Aedes* abundance peaks in southern California (CDPH 2020). These procedures were also performed with *Cx. quinquefasciatus* for comparison and validation of the methods, because this mosquito developed in USDS (Su et al. 2003) and small containers (Reisen et al. 1990) in southern California. All trials were conducted in an insectary with temperature and humidity regulation (27° C and 70% RH). To minimize the potential bias of mosquito colonization on behavior and fitness, all experiments described above used F_1 *Ae. albopictus* adults from Los Angeles County, and *Ae. aegypti* and *Cx. quinquefasciatus* adults from Orange County. Water from the USDS and flowerpots were collected biweekly from the same seven underground storm drains and three cemeteries, respectively, mixed together by type of water source, refrigerated, and held until use. Field-collected flowerpot water was used as the positive control.

RESULTS AND DISCUSSION

Oviposition preference

Aedes albopictus mosquitoes laid significantly more eggs (Tukey-Kramer HSD test, $P < 0.001$) in USDS water than in flowerpot water, whereas the number of eggs in USDS water and flowerpot water was not statistically different for *Ae. aegypti* (Tukey-Kramer HSD test, $P =$

Table 1.—Relative comparisons of oviposition preferences, egg hatching and pupation rates of *Ae. aegypti, Ae. albopictus, and Cx. quinquefasciatus* mosquitoes in USDS water.

Mosquitoes	Egg Laying	Hatching	Pupation rate
Ae. aegypti	+ +	+ + +	0
Ae. albopictus	+ + +	+ +	0
Cx. quinquefasciatus	+ + +	+ + +	+

0.25). Therefore, USDS water was attractive to *Ae. albopictus* for egg laying when only two substrates were offered, whereas *Ae. aegypti* showed no preference when the two oviposition substrates (flowerpot water and underground water) were offered.

Egg hatching

The percentage egg hatch of *Ae. aegypti* in USDS water was 38%, similar to 43% in the flowerpot water. *Aedes albopictus* showed a percentage hatch of 18% in USDS water, significantly greater than 0.1% in flowerpot water (Wilcoxon test, $P = 0.002$). Together, these results suggest that underground water is both attractive for oviposition by gravid females and supportive for egg hatching, for these invasive *Aedes* mosquitoes.

Larval survivorship

Larval development of *Ae. aegypti* and *Ae. albopictus* in USDS water was retarded, and no larvae successfully pupated. In contrast, larval survivorship of *Ae. aegypti* and *Ae. albopictus* in the control (flowerpot water) was 94% and 95%, respectively, and larva-to-pupa duration averaged $<$ 6 days. These results suggest that the USDS water quality was not conducive for invasive *Aedes* larval development.

Overall, *Ae. aegypti* and *Ae. albopictus* were attracted to the USDS water for oviposition. Although *Ae. aegypti* and *Ae. albopictus* eggs hatched at greater or similar rates in the USDS water compared to the flower pot water (38 vs. 43% for *Ae. aegypti*, and 18 vs. 0.1% for *Ae. albopictus*, respectively), larvae did not develop successfully into pupae (Table 1). In contrast, *Cx. quinquefasciatus* successfully completed its entire immature life cycle in the USDS water (Table 1). The low dissolved oxygen, high electrical conductivity, and high salinity found in USDS water (Su et al. 2003), along with various petrochemicals washed in from roadways, all could have adversely affected the growth of invasive *Aedes* larvae.

CONCLUSIONS

Due to their widespread distribution, temporal stability, and favorable micro-environmental conditions, underground storm drain systems in southern California provide an extensive source for *Cx. quinquefasciatus* and potentially, invasive *Aedes* mosquitoes. Our study using field-derived adult mosquitoes demonstrated the attractiveness of USDS water for oviposition and was supportive of egg hatching for *Ae. aegypti* and *Ae. albopictus*. However, USDS water was not conducive to larval development and no larvae pupated for both species. These findings indicated that the USDS would not constitute a significant breeding source for invasive *Aedes* mosquitoes in southern California. However, it is important to compare these findings to ecological conditions of underground stormwater habitats in other areas where *Ae. aegypti* and *Ae. albopictus* are found.

ACKNOWLEDGEMENTS

We would like to thank San Gabriel Valley Mosquito and Vector Control District for their generosity of providing *Ae. aegypti* eggs. We also want to acknowledge the Pacific Southwest Center for Excellence in Vector-Borne Disease Training Fund and the UC Irvine Chancellor's Fellow Fund for financial support.

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Software development doesn’t have to be hard: One District’s path to a new data collection and management system for mosquito and vector control surveillance

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Introduction

During the development of the Shasta Mosquito and Vector Control District’s (District) new data collection system named VeeMac (Fig. 1) , project management and communication became essential with the development team and the District due to the developers working in different locations from each other and the District. To formalize communication and collaboration between District staff and the developers, we searched for free or low-cost tools to ensure consistency. By selecting and implementing software we were able to simplify and increase our development time with the VeeMac developers, improving our data migration experience.

Methods

The District selected Microsoft Excel™, within their Microsoft 365™ online platform, as a shared spreadsheet on which all staff and testers could log issues. This spreadsheet was organized to allow for categorization of the issue thereby helping the developers focus their efforts. For project management, we used Trello™ online to stage development goals. This “kanban” style tool, allowed the team to have real-time communication and full transparency of work. Work items and development issues are represented visually on a “Kanban” board, allowing the team members to see the state of each piece of work at any time (Fig. 2). For a more large-scale scheduling tool, we created a development schedule with Microsoft Office Timeline™ (Fig. 3). Finally, we used Zoom™ teleconfer-

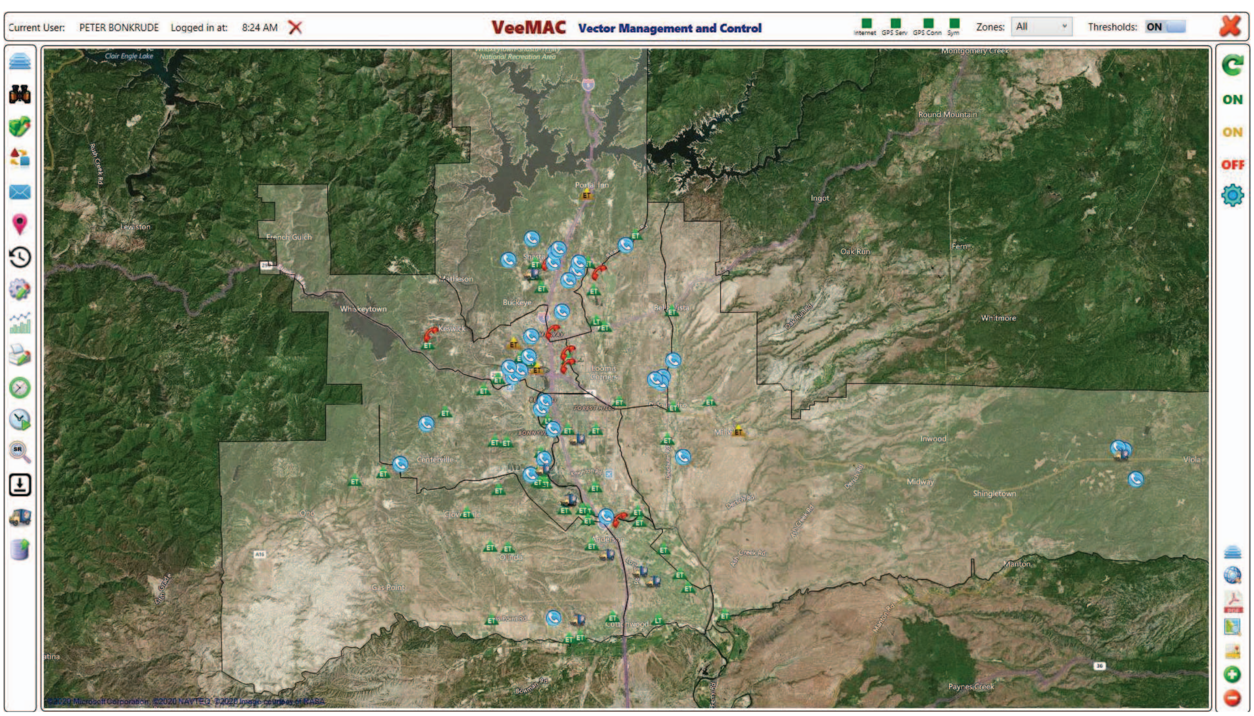

Figure 1.—VeeMac Dashboard

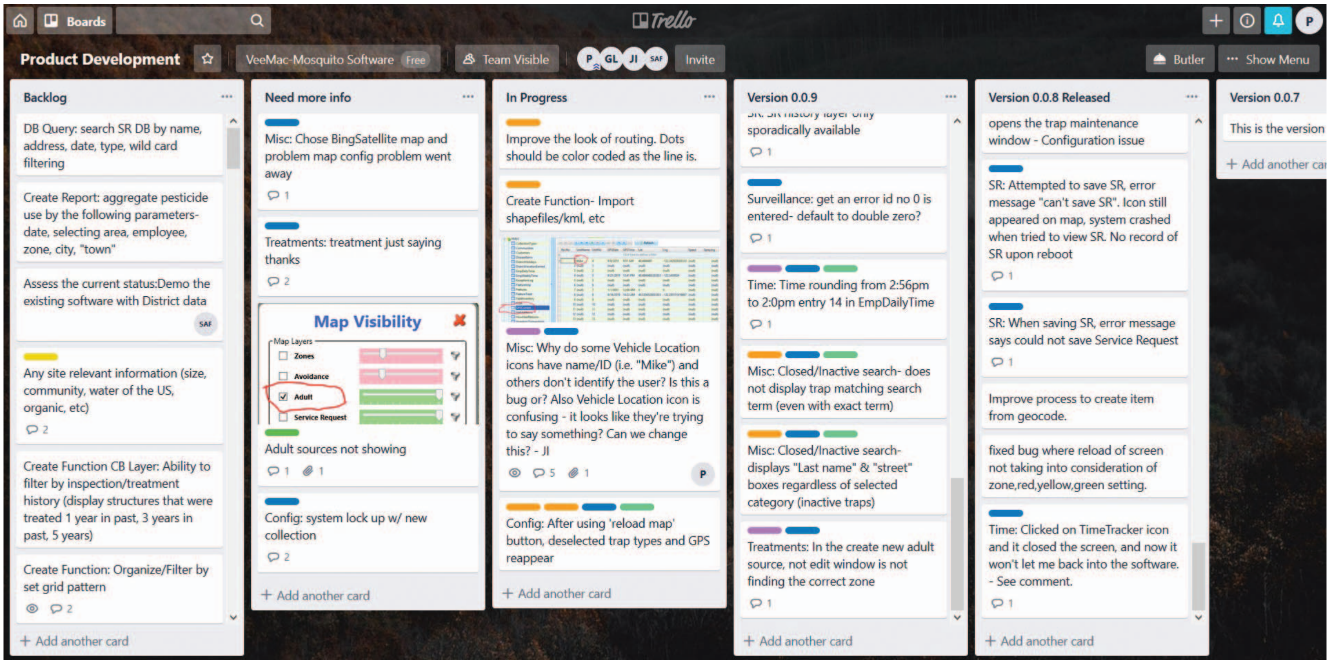

Figure 2.—Trello Board

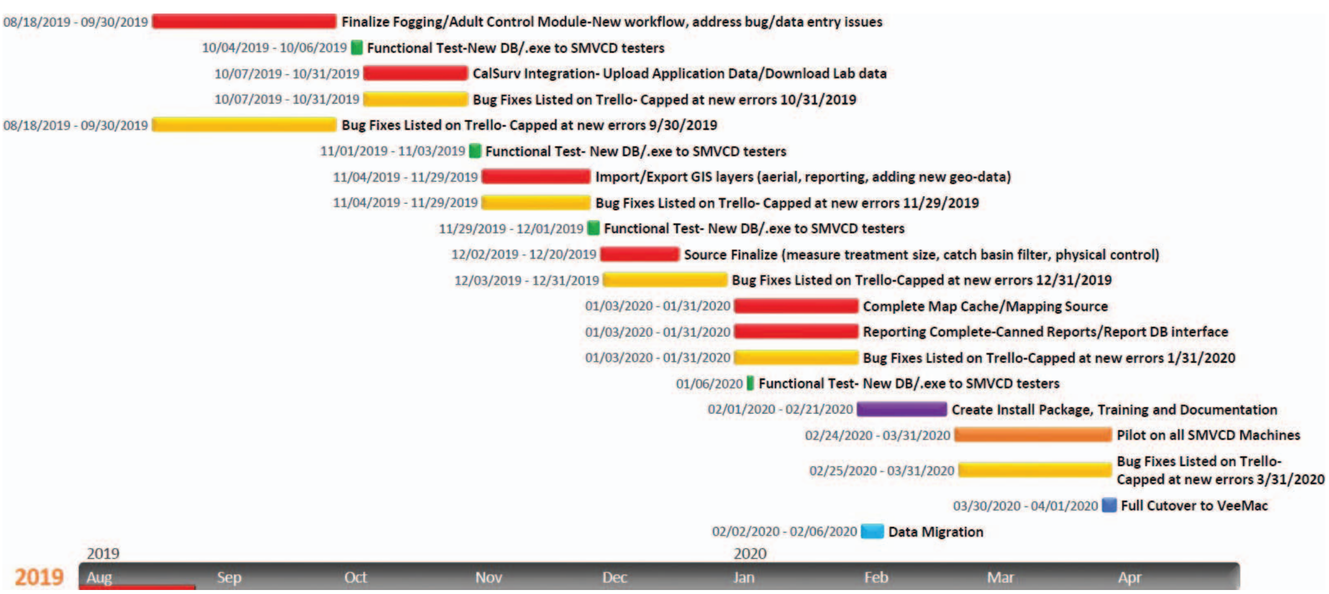

Figure 3.—Project Development Schedule

encing software to meet every two weeks to discuss development and work through issues.

Results and Discussion

Implementing these communication tools and creating a feedback looped helped to involve all staff in the development of the data collection software and ultimately led to more informed process. With little expense, we were able to craft a largely successful strategy that helped bring our project to completion with little additional expense.

Acknowledgements

Thank you to the Staff and Board of Trustees of the Shasta Mosquito and Vector Control District.

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Control of *Aedes aegypti* in Underground Storm Drain Systems in Los Angeles County

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Abstract

Since 2014, the Greater Los Angeles County Vector Control District (GLACVCD), has experienced the introduction, rapid expansion, and establishment of *Aedes aegypti* populations. In addition to the variety of residential and industrial breeding sources exploited by *Ae. aegypti*, larval collections from the expansive network of underground storm drain systems (USDS) beneath Los Angeles and surrounding communities have resulted in the need for modification and revaluation of control strategies. We examined the effectiveness of two sequential USDS field trials of VectoBac® WDG for controlling mosquito populations, most notably *Ae. aegypti*, across several communities of both the San Gabriel and San Fernando Valleys of Los Angeles County. Treatments were evaluated through pre- and post-treatment sampling of adult mosquitoes at test sites using non-baited CDC style CO_2 traps. Our results indicate that VectoBac® WDG was effective at controlling mosquito populations in USDS when applied appropriately

Introduction

The complex network of underground storm drain systems (USDS) which lie beneath Los Angeles and its surrounding communities contribute to the overall mosquito abundance in these areas (Kluh et al. 2002). For the past two decades, the Greater Los Angeles County Vector Control District (GLACVCD) has had resources in place dedicated to mitigating the impact of USDS on mosquito populations within its jurisdictional boundaries. The USDS program at GLACVCD initially was founded in response to the threat of West Nile Virus, focusing control efforts almost exclusively on *Culex* species. However, the introduction and establishment of invasive *Aedes* mosquito species within the greater Los Angeles metropolitan area (Metzger et al. 2017), and recent detections of *Ae. aegypti* in portions of the USDS, have collectively provided the impetus for the modification and reevaluation of control strategies.

Currently, control efforts in the USDS at GLACVCD rely upon applications of a larvicidal mixture of VectoBac 12AS® (active ingredient: B*acillus thuringiensis israelensis (Bti)*) and VecoLex WDG® (active ingredient: *Lysinibacillus sphaericus (Ls)*) mixed at rates of 0.024 l (8 fl.oz.) and 0.45 kg (1 lb.) per acre, respectively (Kluh et al. 2001). Given that *Ae. aegypti* is highly refractive to the toxic effects of *Bs* (Wirth et al. 2000; Park et al. 2010), an alternative larvicide containing only the active ingredient *Bti* is preferred. Our paper examines field trial results evaluating the effectiveness of USDS applications of VectoBac WDG to control *Ae. aegypti*.

Materials and Methods

Two sequential USDS trials were conducted to evaluate the effectiveness of VectoBac® WDG (*Bti*, Valent BioSciences LLC, Libertyville, IL.) in controlling mosquito populations, most notably *Ae. aegypti*, across several communities of both the San Gabriel and San Fernando Valleys of Los Angeles County. In Trial 1, four unconnected USDS test sites were selected in City of Commerce (Fig. 1). Applications of VectoBac® WDG were made to each of the test sites at a rate of 8 oz/acre diluted at 4 oz/gal. Larvicidal applications were made using the GLACVCD USDS Spray Wand (Fig. 2; schematics available upon request). The USDS Spray Wand is outfitted with an Amflo® venturi mixing valve (P/N 230-D, Plews & Edelmann LLC, Dixon, IL.) where compressed air, supplied at 100 psi, aerosolizes the pesticide. Once aerosolized, the larvicide is then applied to the USDS through the keyway in the maintenance hole cover (Fig. 3), precluding removal of the cover. The pesticide flow rate was regulated by setting the venturi aperture to 1/8 turn. All maintenance holes on each of the test systems were treated to ensure complete coverage by the larvicide applications.

Treatment effectiveness of VectoBac® WDG was assessed through pre- and post-treatment sampling of adult mosquitoes at test sites using non-baited CDC style CO_2 traps. Post-treatment sampling was conducted at 5-7 day intervals. Traps were suspended from the top rung of maintenance hole access ladders, beneath the maintenance hole cover. The duration of control was assessed by monitoring trap contents for overall abundance, with emphasis on the presence of male mosquitoes. Presence of male mosquitoes in the collections indicate the waning

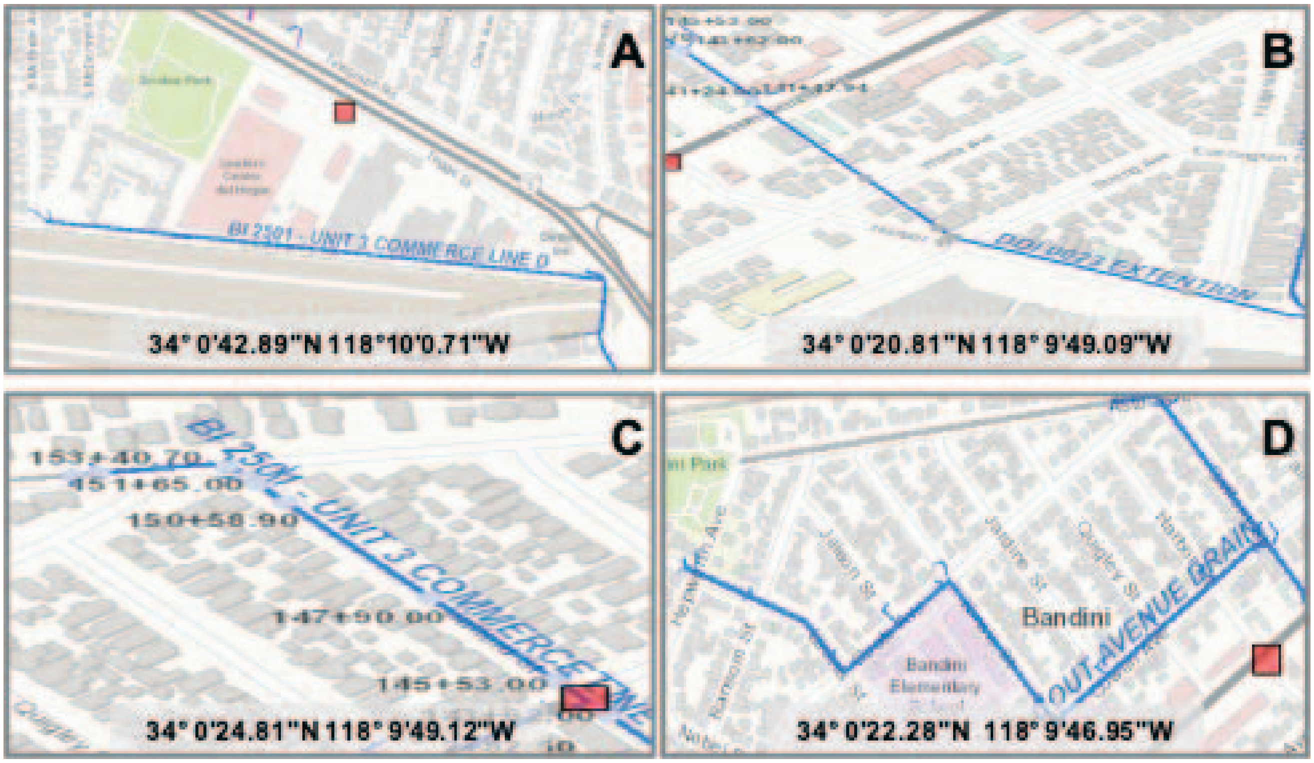

Figure 1.—Trial 1 test sites: Triggs System (A), Harbor System (B), Gafford System (C), and Atlantic System (D).

effectiveness of the larvicidal material and that adult emergence has resumed within the underground system.

In Trial 2, four additional test sites were selected in the communities of North Hills, Canoga Park, and West Hills (Fig. 4). The application method and pre- and post-treatment abundance sampling were conducted identically to Trial 1. However, in contrast to Trial 1, although the application rate of 8 oz/acre remained unchanged, the dilution for Trial 2 was 2 oz/gal. Spray times were adjusted to compensate for this dilution and achieve the required volume of larvicide.

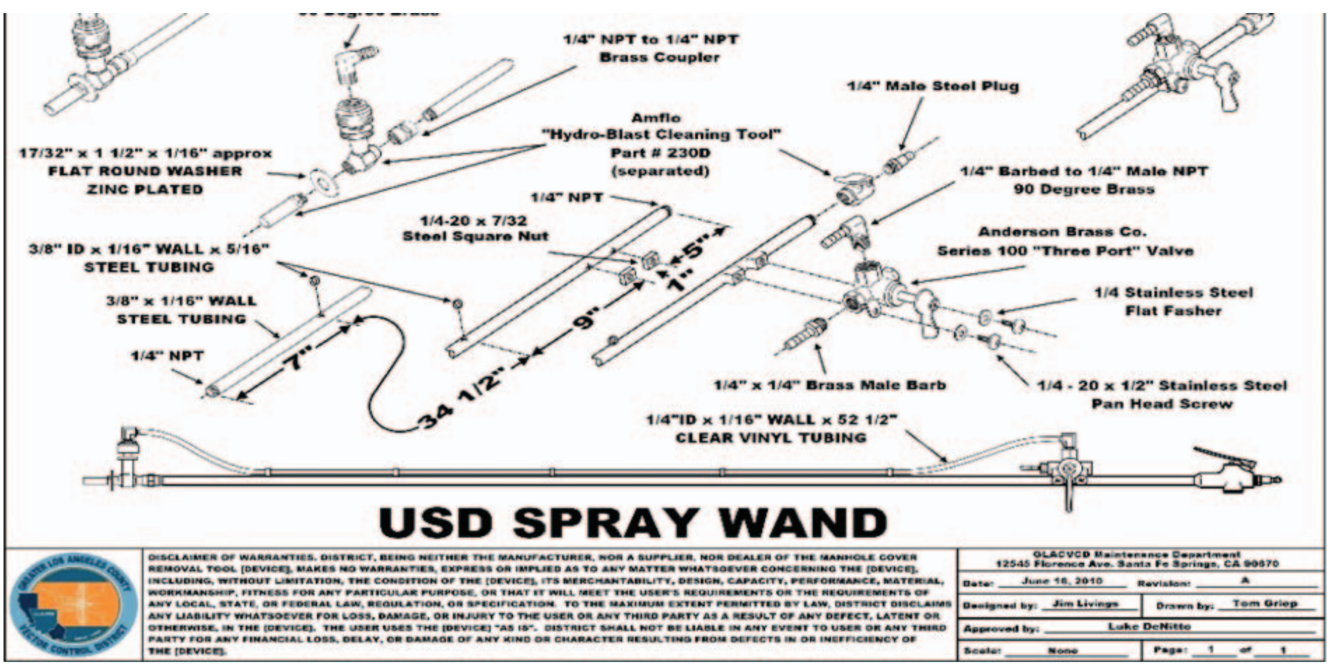

Figure 2.—Diagram showing components of the GLACVCD USDS Spray Wand.

Figure 3.—Larvicidal application using GLACVCD USDS Spray Wand.

Droplet characterization of pesticide aerosols was conducted by placing Kromekote paper cards (3-inch x 5-inch) at 15-foot intervals for a total distance of 60 feet. The cards then were sprayed with VectoBac® WDG tinted with food-grade dye. Sprayed cards were allowed to dry and analyzed using DropVision® AG software (Leading Edge, Fletcher, NC).

Results and Discussion

In Trial 1, the applications of VectoBac® WDG to the USDS test sites yielded a duration of control ranging from 11 to 17 days assessed by pre- and post-treatment trapping. When compared to the VectoBac® 12AS/VectoLex® WDG mixture currently in use, the observed period of control of VectoBac® WDG was far shorter than the 28 days expected with the larvicidal mixture. Droplet characterization compared droplet profiles of both the VectoBac® 12AS/VectoLex® WDG duplex and the VectoBac® WDG field mixtures to determine whether the viscosity of the latter impacted the application equipment to produce appropriate droplet sizes. Droplet analysis demonstrated that VectoBac® WDG, when mixed at 4 oz/gal, produced droplets with a significantly larger volume median diameter (VMD) than the VectoBac® 12AS/VectoLex® WDG duplex (Fig. 5). These results indicated that a substantial proportion of the spray volume was lost due to precipitation before reaching the cards downrange.

The droplet characteristics of two additional dilutions of VectoBac® WDG (3 oz/gal and 2 oz/gal) also were compared. During the droplet characterization of the two more dilute mixtures, spray cards at the 15-foot distance became saturated and were exceedingly difficult to read accurately with the DropVision software. As a result, the

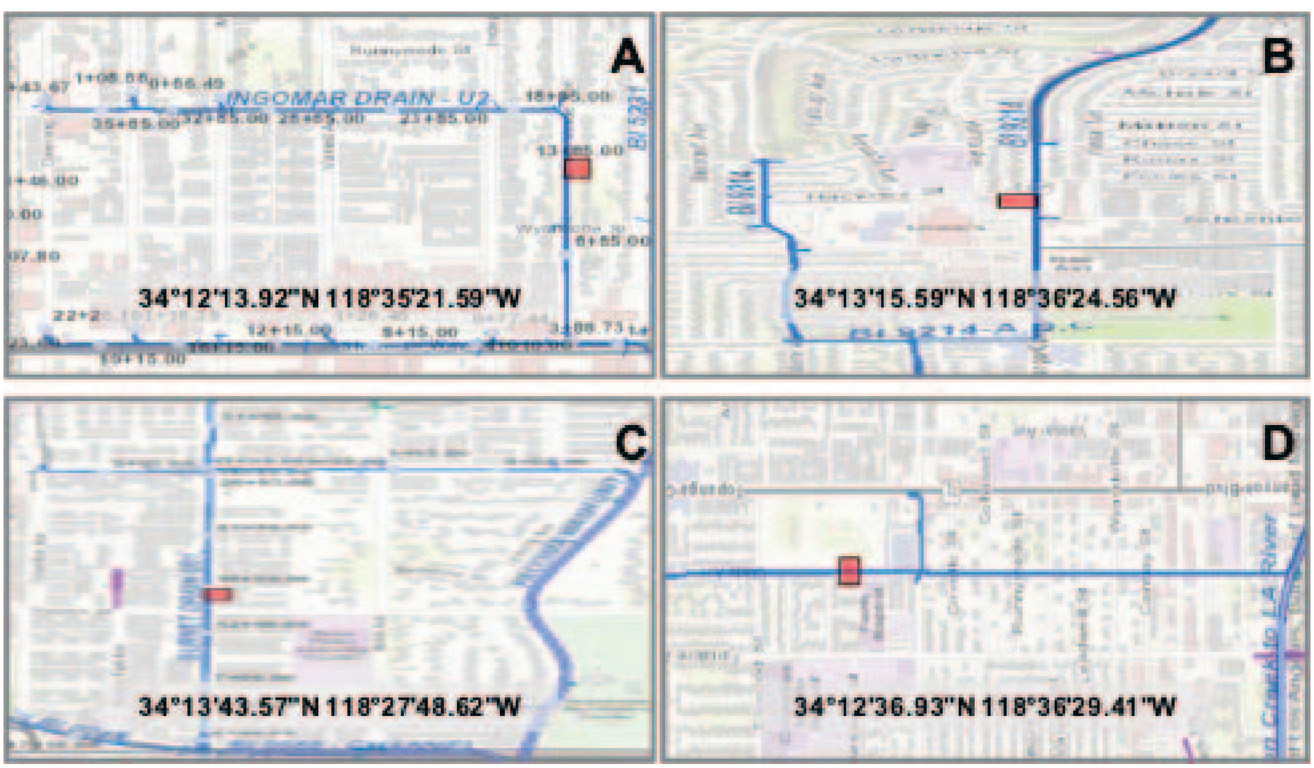

Figure 4.—Trial 2 test sites: Ingomar System (A), Topanga Cyn System (B), Burnet System (C), and Glade System (D).

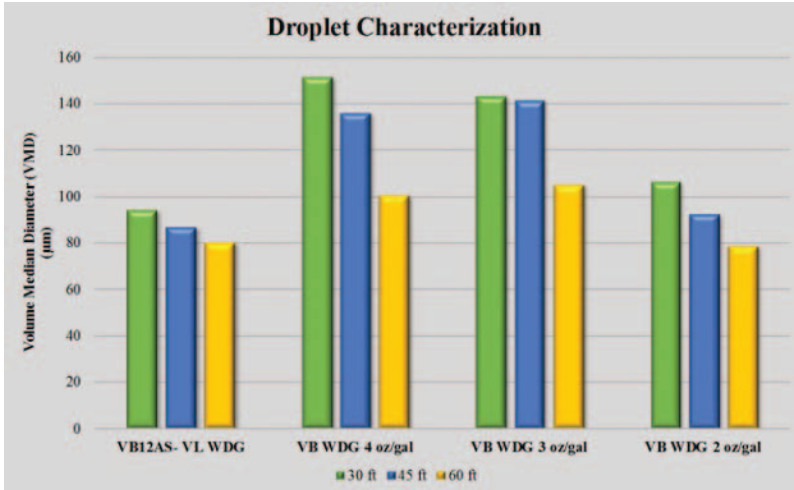

Figure 5.—Volume Median Diameter (VMD) comparison of VectoBac® WDG dilutions to VectoBac® 12AS/VectoLex® WDG duplex.

15-foot cards were omitted from the comparisons. Of the three dilutions of VectoBac® WDG, the droplet profile of the 2 oz/gal most closely resembled that of the VectoBac® 12AS/VectoLex® WDG duplex with regard to VMD, droplet density (drops/cm^2), and volume density (gal/A) (Fig. 5).

In Trial 2, the USDS test site applications of VectoBac® WDG with a dilution rate of 2 oz/gal achieved between 21 to 25 days of control. These results were a marked improvement over the residual control observed in Trial 1. Although increased spray times were required to reach the appropriate volume of pesticide applied at each treatment site, the thinner viscosity of the 2 oz/gal dilution did produce a more consistent droplet profile resulting in more effective treatment.

Conclusions

Overall, the results of these field trials demonstrated the effectiveness of VectoBac® WDG in controlling mosquito populations in USDSs, including *Ae. aegypti*, when applied appropriately. Based on our findings, the GLACVCD will include the VectoBac® WDG formulation as a supplemental larvicide in its USDS program to treat systems where *Ae. aegypti* have been detected. Should the detection of *Ae. aegypti* within the USDS of the GLACVCD service area expand, VectoBac® WDG will replace the VectoBac® 12AS/VectoLex® WDG duplex currently being used. Further evaluation of VectoBac® WDG will be conducted in broader field trials to optimize applications before the product is broadly used.

Acknowledgements

We thank Peter DeChant and Stephanie Whitman of Valent BioSciences, for their technical assistance. We would also like to thank the USD Program staff of GLACVCD, especially Chris Dwyer, David Olmos, Shaun Maki, and Peter Mortley for their efforts during the course of the field trials.

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Initial Detection and Response to *Aedes aegypti* in Sacramento County

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Introduction

Aedes aegypti is an invasive mosquito to California that has become established in the Southern and Central regions of the state, but until recently has not been detected in Northern California. Upon detection of this mosquito in Sacramento County, the District implemented an aggressive campaign to contain this infestation. This campaign extended from August to October with the goal of lowering the number of properties with detections.

Methods

To help suppress the *Ae. aegypti* population, the District used tools available through its existing Integrated Vector Management program, with slight modification to address the biology and behavior of this species. A door-to-door campaign was initiated in the area of concern resulting in public education, source reduction, larviciding and adulticiding. In addition, the laboratory deployed BG Sentinel traps in the area at a ratio of one trap per six houses to ascertain the scope of the infestation. Larvicide treatments via VectoBac WDG were performed by both backpack and truck mounted applications. Adulticiding with Deltagard was done in a similar manner.

Results and Discussion

On August 28th, 2019, the Placer County Mosquito and Vector Control District notified the District of an *Aedes aegypti* detection in Placer County 700 feet North of the Sacramento/Placer County boarder. The next day field technicians were deployed on the Sacramento County side of the boarder in a door-to-door campaign which resulted in the first detection in Sacramento County. This detection was a watering can that contained eggs through adults. Concurrently, an in-depth surveillance program was initiated to determine the scope of the infestation. Trapping led to the identification of 15 properties with adults. Upon detection, the District implemented larval and adult control measures and then trapped the area again to determine the impact of these control measures. Of 12 properties available for re-trapping, four were positive for *Ae. aegypt*. Of these four, two had a decrease in the number collected, one trap had two more mosquitoes when re-trapped, and one trap stayed the same. These surveillance and control measures remained in place and then were deployed in the same manner when a second area of concern emerged, with similar effects.

Conclusions

Both the aggressive treatment and trapping strategy allowed the District to quickly reduce the number of detections in two areas of concern. Moving forward the District will be investigating different approaches to dealing with invasive mosquitoes including expansion of staff as well as exploring the release of sterile males.

Acknowledgments

We would like to thank Placer Mosquito and Vector Control District for coordination of efforts, Chris Barker and his lab for additional traps and ADAPCO and Valent Biosciences for the expedited shipping of materials needed for this response.

Evolving *Aedes* control methods and service request management in the face of changing public expectations

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Abstract

The rapid expansion of the Aedes mosquitoes from the southern part of our District to the San Fernando Valley over the last five years has changed the way we process service requests, manage site inspections and address public expectations. Service requests may be submitted via phone or our website, screening of requests is important to efficiently service residents. The electronic database used encompasses a map interface allowing for proficient distribution of requests based on specialist zones and allows for daily routes to be developed with minimal prep-work.

In the field, a shift in *Aedes* breeding has been seen in areas where residential awareness/education is robust and typical breeding sources have been removed. *Aedes* mosquitoes have shifted into clogged outdoor drains, low-flows, gutters and under grounds. For residential outdoor drains we are recommending: screening, cleaning, weekly treatments with Mosquito Bits or the use of impermeable materials to cover drains during the summer and removing for the winter.

Resident expectations and the demand for barrier spraying and adulticiding is soaring. Some residents are responding poorly to our education/source reduction approach and are unwilling to use repellent or accept that *Aedes* mosquitoes are now going to be a part of California life. Explanation of adulticiding and chemical usage has helped alleviate pushback from some residents.

Efficient service request management is vital, as *Aedes* mosquitoes continue to rapidly expand triggering the growth of service requests. The importance of source reduction/management should be communicated effectively because localized spraying is not a long term solution.

Developing a program to address the breeding of *Aedes* and *Culex* mosquitoes associated with stormwater structures in Los Angeles County

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Abstract

Securing a sustainable supply of water for Los Angeles County has been a top priority as a result of the increasing population. Most of the water is purchased and stored in reservoirs or used to augment ground water reserves to be used as needed. Stormwater capture and use is gaining attention throughout the region as a way to improve water security. Unfortunately, the structures built to capture, clean and store stormwater can also be conducive to mosquito production. This paper describes our process for developing a program to address the control of *Aedes* and *Culex* in stormwater structures.

The Greater Los Angeles Vector Control District (District) currently has an Urban Water Program Manager and two full time Vector Control Specialists working to identify and catalog structures. The District also is working with agencies, contractors and manufacturers to raise awareness of the potential for these structures to produce mosquitoes and ways to prevent this production. Measure W, the Safe Clean Water Act, was approved by Los Angeles County residents and will provide funding for water projects into the foreseeable future, many of which will create habitat for mosquitoes.

The District program is in its infancy, but has the potential to play a significant role in mosquito reduction efforts. We are already seeing progress by raising concerns with agencies, contractors and manufacturers.

Impact of invasive *Aedes* species targeted backyard mosquito control measures on local *Culex quinquefasciatus* populations

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Abstract

The establishment of three container breeding invasive *Aedes* species in Los Angeles County, California since 2011 has altered the demand for services on agencies such as the Greater Los Angeles County Vector Control District (District). The never before experienced level of biting pressure that these daytime biters unleash upon Southern California residents has increased the number of requests for service to the District more than tenfold compared to the pre-*Aedes* infestation monthly call average. Although the overall emphasis of the District's control effort remains on larval control and source reduction targeting traditional *Culex* spp. sources, additional staff and their response to these *Aedes* spp. driven service requests have resulted in the inspection of unprecedented number of backyards within the District's service area. While targeted to reduce *Aedes* spp. populations, these backyard efforts also provide additional *Culex* spp. control above and beyond the standard control of publicly accessible mosquito sources and reported unmaintained swimming pools. This publication examines the impact of backyard source reduction and treatment on district wide *Culex quinquefasciatus* abundance.

INTRODUCTION

Before the introduction of West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) to Southern California in 2003 (Reisen et al. 2004), St. Louis encephalitis virus (*Flaviviridae: Flavivirus*, SLEV) and western equine encephalomyelitis virus (*Togoviridae: Alphavirus* , WEEV) were the mosquito-borne encephalides of concern in the greater Los Angeles area. Different from WNV which first emerged and continued to thrive in highly urban environments with *Culex pipiens quinquefasciatus* Say as primary vector (Kwan et al. 2010), SLEV and WEEV were considered to be rural health problems, mostly of the Central Valley (Reeves et al. 1990). During outbreaks of SLEV in urban Los Angeles the 1980s (Reisen et al. 1992) infected pools of *Cx. tarsalis* Coquillett were detected (Murray et al. 1984) perhaps implicating this species in the urban transmission cycle. Therefore, the District's surveillance program used to be focused on the more natural spaces and *Cx. tarsalis*. In anticipation of the arrival of WNV had to be refocused on conducting most of the routine surveillance throughout the urban areas searching for *Cx. quinquefasciatus*.

The District's service area spans across 1,340 square miles of densely populated urban landscape containing approximately one million parcels. In the pursuit of *Cx. quinquefasciatus* population reduction to prevent WNV transmission, we presumed to have good control of most of the publicly accessible larval sources such as spreading basins, gutters or roadside ditches. But, the extent to which our backyard sources contribute to *Cx. quinquefasciatus* breeding in our neighborhoods, besides the occasionally reported unmaintained swimming pool, has long been an unknown part of the control equation, because the District was lacking the staffing to conduct an assessment of the many hundred thousand residential back yards.

The arrival and spread of the invasive *Aedes* spp. in Los Angeles County (Metzger et al. 2017) and the immense biting pressure they unleashed upon residents, resulted in a tenfold increase of service requests and an unprecedented number of property inspections, along with source reduction and the application of traditional treatment options. Because of the *Aedes* spp. driven property inspections, the importance of these sources in *Culex* production could finally be evaluated on a large scale.

MATERIAL AND METHODS

To evaluate the contribution of backyard breeding to *Cx. quinquefasciatus* abundance within the District's service area, larval samples collected over the course of several years during *Aedes* spp. service requests were identified to species. Samples used for this analysis were collected during 2019.

Adult mosquito abundance data collected District-wide bi-monthly between the beginning of 2014 and the end of 2019 in gravid as well as CO_2 baited traps at 163 locations was used to assess the impact of the property inspection associated source elimination and control efforts triggered by *Aedes* spp. service requests on over all *Cx. quinquefasciatus* abundance.

Linear trendlines were fitted by least squares using Excelsoftware. WNV activity was measured in percent positive mosquito samples. Pools of between 12 and 50

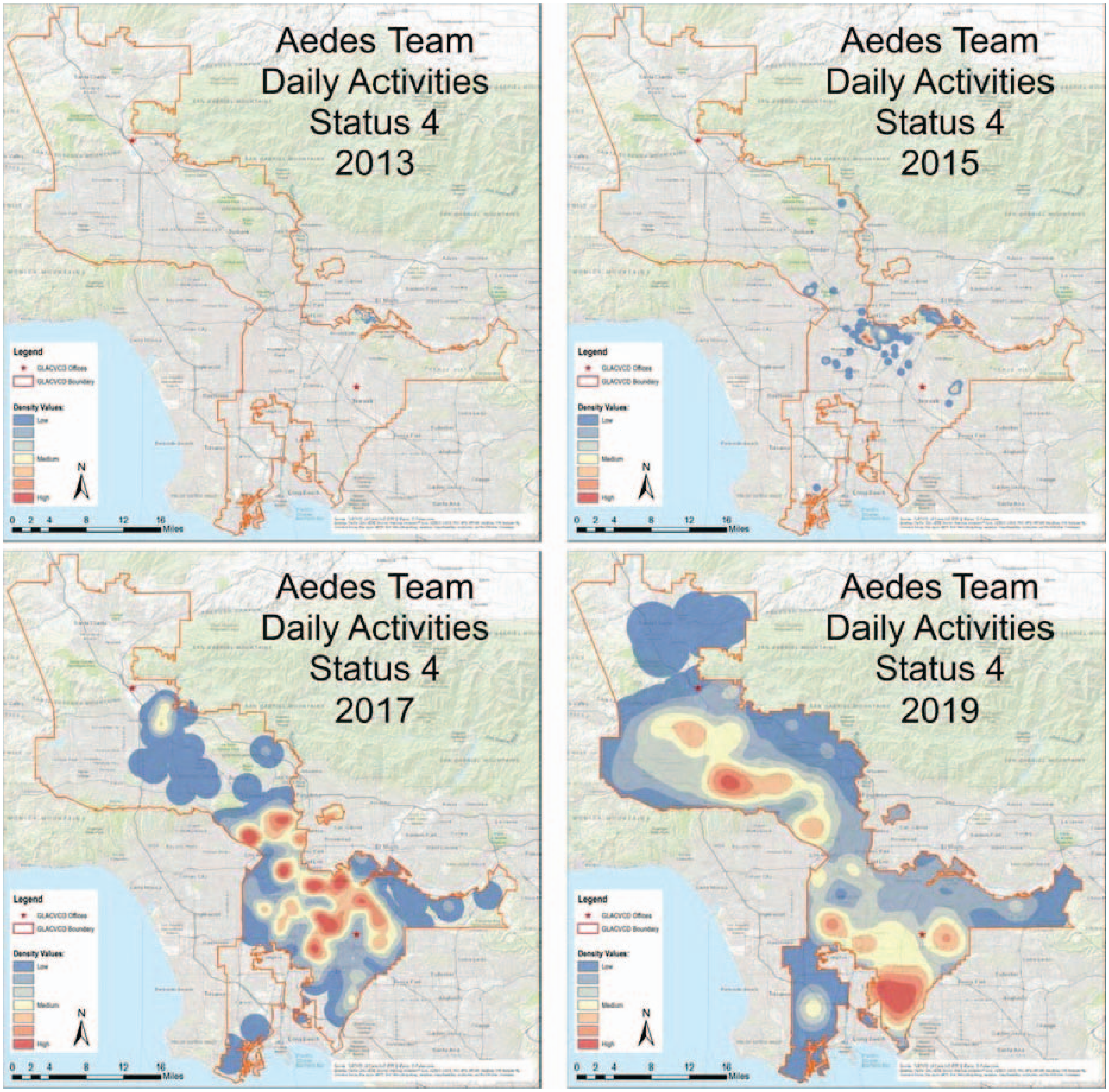

Figure 1.—Heat map of *Aedes* spp. positive property inspections depicting the *Aedes* spp. spread across the District's service area in two year intervals.

female mosquitoes from every site during each trapping cycle were submitted for WNV, SLEV and WEEV testing.

RESULTS AND DISCUSSION

Between 2013 and 2019 *Aedes* spp. have spread throughout almost the entire the District service area (see Fig. 1).

Service requests since the emergence of WNV in Los Angeles County in 2003, increased from normal levels of around 800 per year to close to 2000 in 2004 and just below 3000 in 2008, respectively, during epidemic years. The invasion and expansion of *Aedes albopictus* (Skuse) and *Ae. aegypti* (L.) created a level of biting pressure that drove residents to request mosquito control services at a rate never before experienced, resulting in almost 8000 phone calls in 2019. In response to these phone calls, as well as to 44 imported cases of *Aedes* spp. borne disease, District staff conducted 24,635 property inspections (Fig. 2)

As time permitted during the early part of the season, 496 larval samples were collected and identified to species. Larvae of three *Aedes* species (*Ae. aegypti*, *Ae. albopictus*, *Ae. notoscriptus*), two *Culex* species (*Cx. quinquefasciatus, Cx. stigmatosoma*) and one *Culiseta* species (*Cs. incidens*)

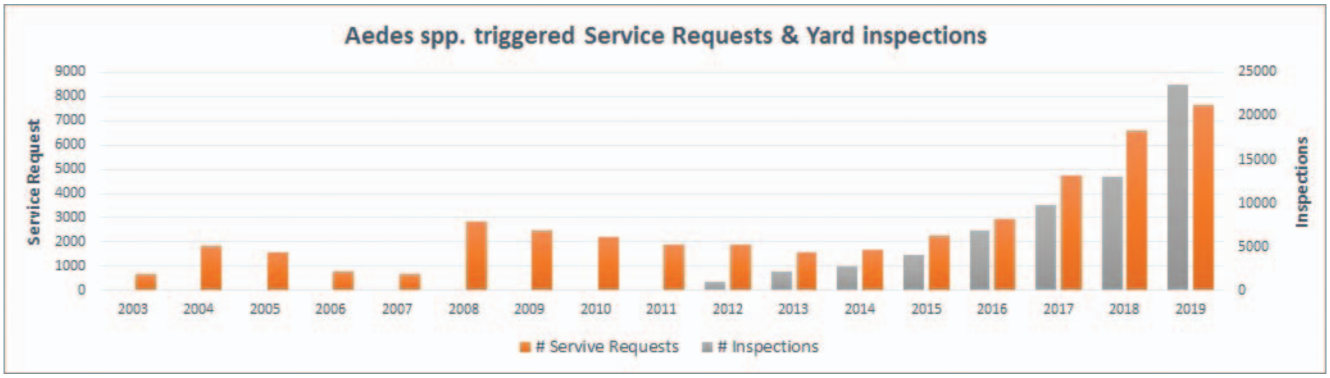

Figure 2.—Number of *Aedes* service requests and yard inspections during 2003-2019.

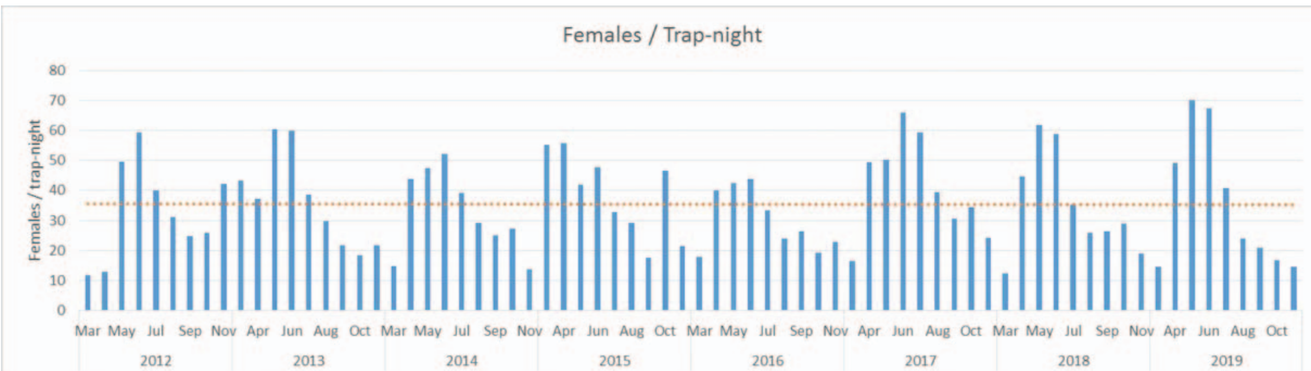

Figure 3.—Average number of *Cx quinquefasciatus* females per CO_2 and gravid trap collected District-wide during 2012 – 2019 (n = 163 locations). Red dashed line shows the trend (least squares fit) remained constant.

were recorded. Despite the fact that the District's current larval sampling favored the detection of *Aedes* spp. larvae in the muck at the bottom of the source over potential *Culex* spp. sampling at the surface, 223 samples (45%) contained *Cx. quinquefasciatus* larvae, indicating that backyard sources were substantially contributing to overall *Cx. quinquefasciatus* production (see Table.1).

These data raise the possibility that the almost 63,000 *Aedes* spp. generated property inspections and resulting treatments, in addition to the continuation of the Districts routine *Culex* spp. sources control, may have had a

Table 1.—Mosquito species composition in 496 larval samples during 2019. Collections made from positive containers.

2019 Larval Samples		
Species	# of Samples	%
Negative	13	2.6
Ae. albopictus (only)	6	1.2
Ae. aegypti (only)	226	46.6
Cx. quinquefasc iatus (only)	121	24.4
Cx. quinquefasciatus (present)	223	45.0
Aedes spp. (only)	237	47.8
Cu/ex spp. (only)	171	34.5
Aedes spp.& *Cu/ex* spp.	230	46.4
Total # of Samples	496	

measurable impact on *Cx. quinquefasciatus* abundance District wide. The larval data indicated that close to 50% of source reduction and treatment efforts targeting *Aedes* spp. reduction also impacted the *Cx. quinquefasciatus* population. However, district wide *Cx. quinquefasciatus* abundance data over the years since the detection of *Ae. albopictus* and *Ae. aegypti* did not show a marked change in female counts per trap-night (Fig. 3).

Given that there are many hundreds of thousands of residential parcels in the District service area, it is not surprising that treatment of 60,000 of them did not markedly change area wide abundance levels. In 2013 and 2014, however, with only one invasive species detected in a small area (see Fig. 1, 2013), all of the *Aedes* spp. control efforts of the agency were focused on the City of South El Monte. In those two years over 5,000 property inspection were conducted in the approximately 3 square mile area containing 4,700 housing units. Every property in the city was likely inspected at least once and the problematic ones visited multiple times. Trap-counts in the South El Monte area show *Cx. quinquefasciatus* trending downward during those years, with a final reduction of almost 50% (Fig. 4). With the arrival of *Ae aegypti* and its rapid spread, these focused control efforts could not be sustained with

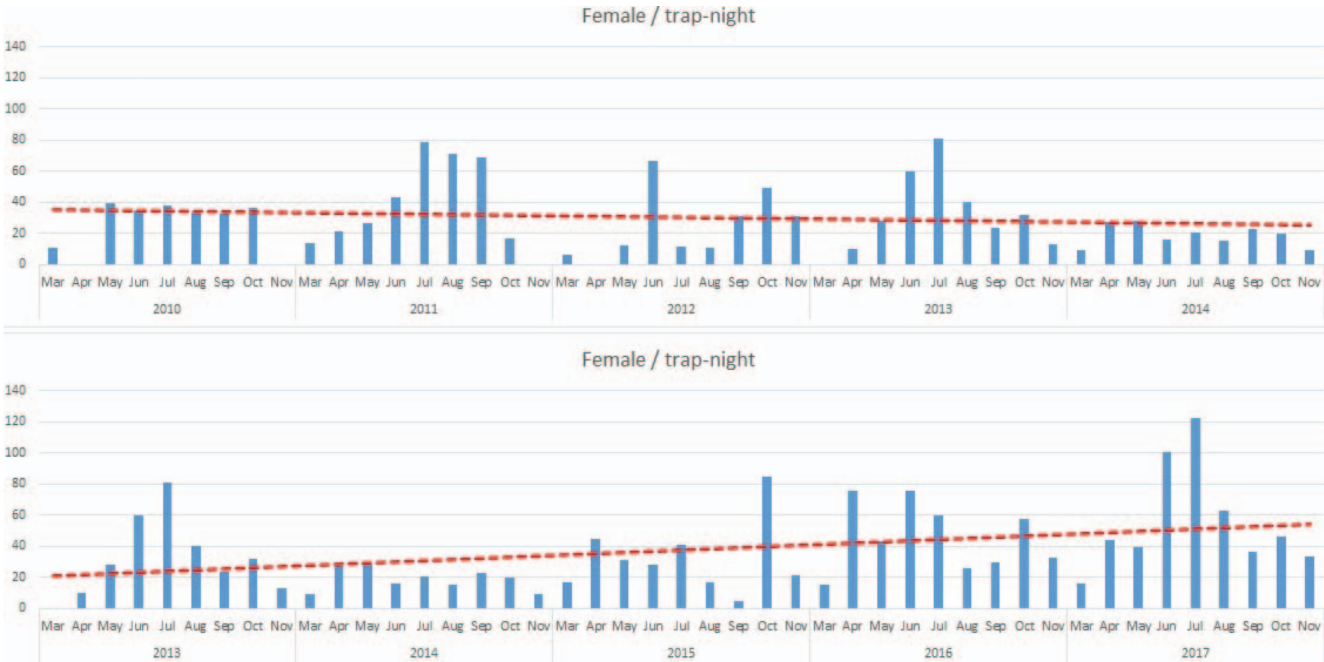

Figure 4.—Average number of *Cx quinquefasciatus* females per CO_2 and gravid trap collected in South El Monte during 2010-14 (top) and 2013-2017 (bottom). Red dashed lines show changes in the 5 year average calculated using least squares fit.

available resources and *Cx. quinquefasciatus* trap counts have gradually increased (see Fig. 4).

A gradual reduction of *Cx. quinquefasciatus* abundance also was observed in the City of Commerce, after *Ae. aegypti* was found there during the summer of 2014 and efforts were made to contain the spread of this second invasive species. Numbers were reduced by 20%, but similar to South El Monte, this reduction was not sustainable (Fig. 5).

Although the response to eliminate and contain invasive *Aedes* spp. has strained District resources, the control of the principal vector of WNV in the Los Angeles Area, *Cx.*

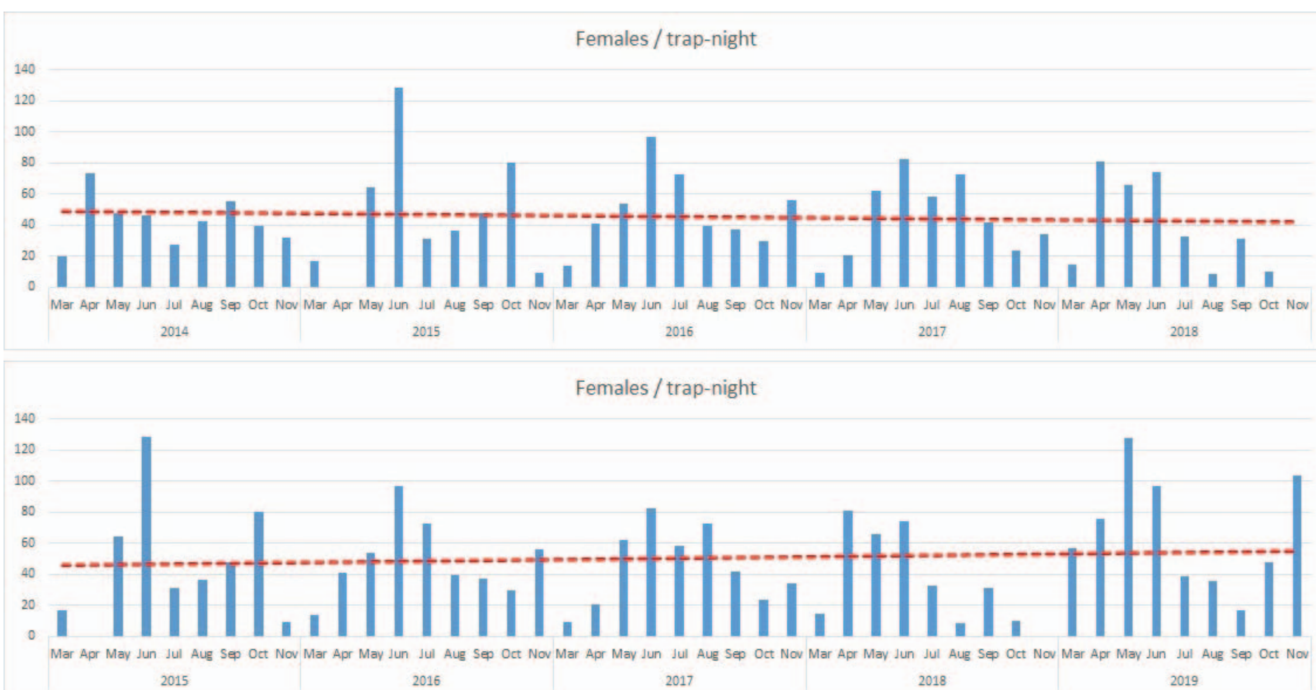

Figure 5.—Average number of *Cx quinquefasciatus* females per CO_2 and gravid trap collected in Commerce during 2014-18 (top) and 2015-2019 (bottom). Red dashed lines show changes in the 5 year average calculated using least squares fit.

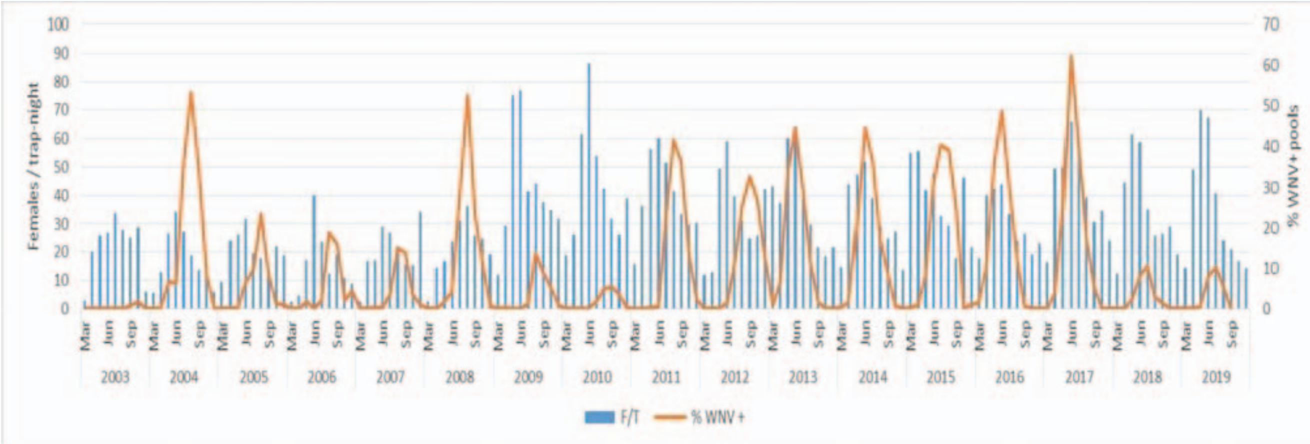

Figure 6.—Average number of *Cx. quinquefasciatus* females per trap night and percentage of mosquito pools positive for WNV during 2003-2019.

quinquefasciatus, remains highest priority, because human cases occur annually, whereas evidence for autochthonous transmission of dengue, Zika or chikungunya viruses have yet to be detected. Since WNV introduction into southern California in 2003, an examination of *Cx. quinquefasciatus* abundance indicated that changes in WNV activity was independent of vector abundance (Fig. 6). Years of high abundance and low virus activity were just as frequent as years of low abundance with high WNV levels.

CONCLUSION

Larval sampling efforts during 2019 showed that residential larval sources were an important contributor to overall *Cx. quinquefasciatus* abundance. Intense property-oriented control efforts can decrease *Cx. quinquefasciatus* abundance, but the percentage of properties treated has to be too high to be sustainable under realistic District staffing conditions. Although there seems to be no clear correlation between zoonotic levels of WNV activity as measured in percent WNV positive mosquito samples and *Cx. quinquefasciatus* abundance over the years, it can be assumed that lower mosquito numbers have translated into fewer mosquito bites and consequently a reduction of human infection risk.

ACKNOWLEDGEMENTS

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The Importance of Inspecting and Educating Simultaneously When Responding to *Aedes* and *Culex* Service Requests

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Introduction

In 2011 Greater Los Angeles County Vector Control District (District) positively identified invasive *Aedes albopictus* within its district boundaries. Just a few years later in 2014 the District identified two additional invasive *Aedes* species within its boundaries, *Aedes notoscriptus* and the now very prevalent *Aedes aegypti*. Since 2013 the District has had an alarming increase in the number of Service Requests from the public, increasing as much as 61% from one year to the next (Fig. 1). An increased number of Service Requests affects the response time, which is measured as the time from when the Service Requests are submitted to the time contact is made with the resident. Ideally, response time does not exceed 48-72 hours, however, sustaining this rapid response can be a challenge as Service Request numbers increase. As response time continues to increase, the pressure on staff to be time efficient also increases. This paper examines the District's strategy and control methods, to shorten response time and determine what actions are most important and effective.

Challenges

Traditionally, many districts have relied on inspections and source reduction when responding to Service Requests and have been successful with the resources available. However, with a rapid increase in Service Requests over the last few years due to the introduction of invasive *Aedes* species, a period of decreased rainfall that resulted in residents watering more frequently followed by a period of increased rainfall, high temperatures and residents moving and sharing contaminated items, the District had to re-examine its response strategy. *Aedes* have been a major contributing factor to Service Request increases as they have rapidly spread throughout the District (Fig. 2) The District may not have control over the weather, but it does have control of its actions and how it responds to these new challenges.

Response Plan

The District's response has been to increase staff in multiple departments, not just Operations. The District holds meetings in which ideas are shared of what has worked, what didn't work and what changes need to be made for the following season. Different departments within the District discuss how they can become more efficient. *Aedes* team members are assigned specific zones and primarily respond to Service Requests within these zones which allows them to become very familiar with their area. Assignments remain flexible and can be modified to provide assistance among zones when necessary. Clear documentation and communication provide shared information among all District staff. Community outreach continues to do excellent work with the public.

Service Response Methods

Inspections and source reduction are excellent methods of mosquito control that may save time if educating the resident is omitted. However, the resident or business owner typically does not know how to maintain their property. They lack the knowledge of which mosquito species were present and that drought resistant eggs may still be present after mitigation. (Fig. 3) They typically don't know the potential health risks of allowing mosquitoes to develop on their properties. Therefore, the resident is likely to continue to have sources on their property and will not prevent future mosquito development. This rapid control approach therefore results in return visits or the impression that the response did not produce a permanent solution.

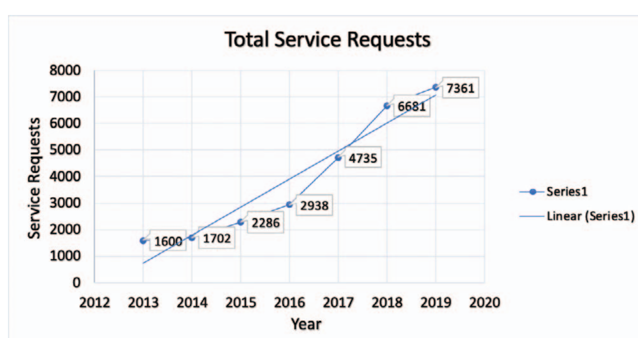

Figure 1.—Total number of Service Requests received by Greater Los Angeles County Vector Control District from 2013-2019.

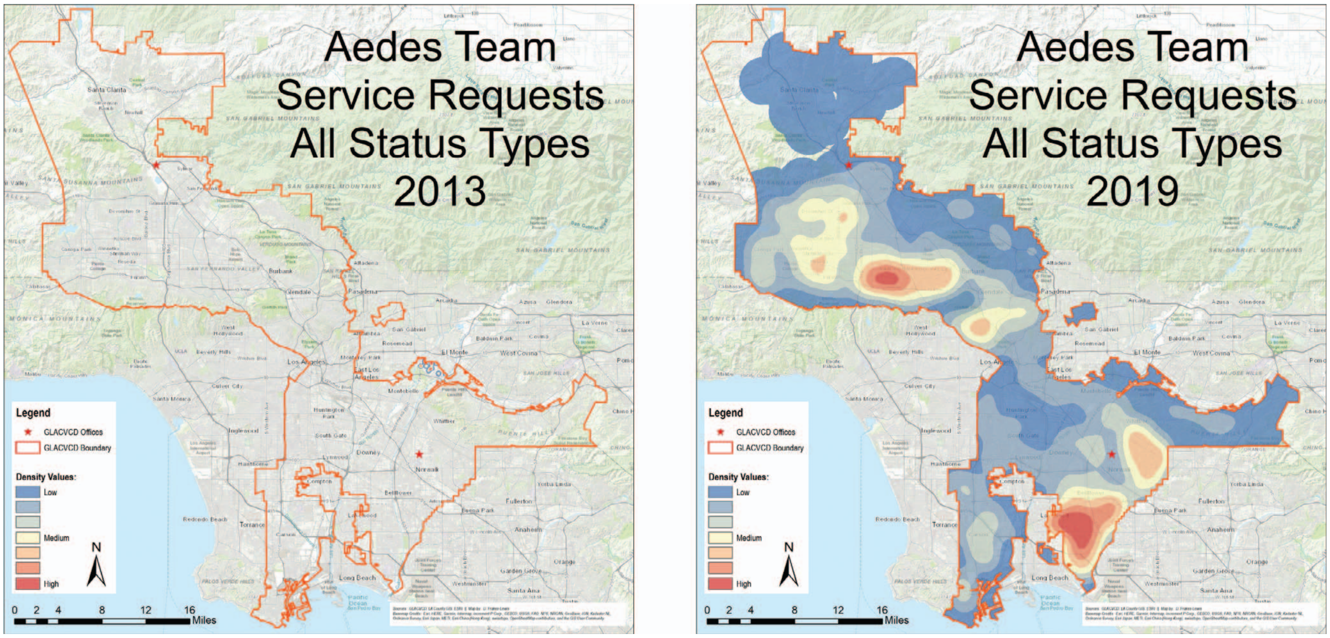

Figure 2.—Total number of *Aedes* Service Requests received by Greater Los Angeles County Vector Control District 2013 vs. 2019.

The benefit to educating the public is that they learn how to protect themselves and their families with the use of repellents and that they can be proactive on their own property with tipping stagnant water and removing unnecessary containers. However, sources may be missed. For example, in many properties the resident stated they had already done research, had conducted their own inspections and wanted the District to provide treatment. During the inspection that followed, some sources frequently were overlooked on their property, either because they were unaware it was there or they were unaware mosquitoes could develop there. Commonly missed sources include, but are not limited to, improperly sealed rain barrels, drains, water reservoirs of fountains, plant trays, pots with no drainage, double potted plants and plant cuttings. Another common outcome was that the resident was unable to remove the source themselves. Typically, it was a source that was too heavy, such as a rain barrel filled with water or a very large potted plant that did not have drainage holes, was over watered, or had a plant tray underneath holding water. Additionally, in some situations the source was located at a neighboring property and the homeowner of that property was unaware of the mosquito activity because they lacked the knowledge to prevent mosquito development or they did not spend much time outdoors and were not bitten by the mosquitoes. Conversely, the approach of only providing education without inspecting properties, conducting source reduction or treatments usually resulted in the public thinking the District did not provide an actual service and did not take any action.

Figure 3.—*Aedes* drought resistance eggs found on a super absorbent cloth in a residential property.

Doing both the inspection and education can be time consuming if not done concurrently. Not all Service Requests are the same and there isn't exactly a 'one size fits all' approach. Some situations required more focus on the inspection, while others required more focus on education, but it is important to incorporate both. For example, a resident that is not very mobile may require you to focus more on the inspection and source reduction, whereas a resident that has dedicated a lot of their time to their landscaping may require you to focus more on education and the importance of properly sealed rain barrels. It is crucial to know your audience.

On the educational side, the District tries to focus on a few key points. To save time we implemented the 'Walk and Talk' practice which means bringing the resident along during the inspection. This way source reduction also enables education, showing the resident the source. Not everyone is familiar with the mosquito life cycle or what exactly to look for. Distribute pamphlets so residents will

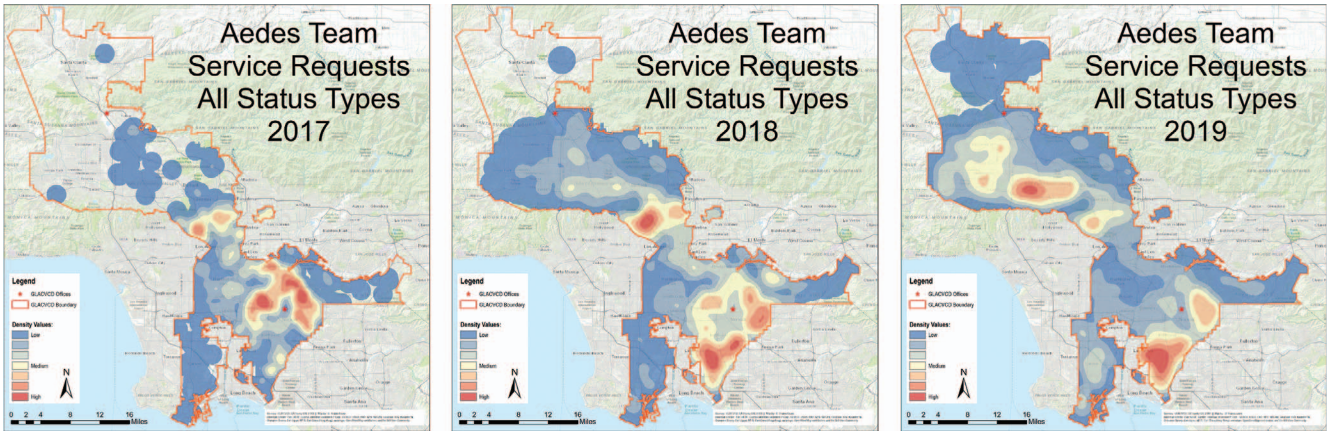

Figure 4.—Decreased vector control demand in areas within Greater Los Angeles County Vector Control District boundaries 2017 vs. 2018 vs. 2019.

be able to read information in detail after the inspection. For example, point out the key differences in *Aedes* and *Culex* mosquitoes and their behavior; this gives the resident a better understanding of what they are experiencing and they become more engaged in the interaction by asking questions. Make it a lifelong learning experience, whether trying to change the resident's expectation for treatment with pesticides or trying to ensure individuals that are against the use of chemicals, that physical and biological control measures are also an equal and important part of Integrated Vector Management. Typically, neighbors, friends and family are experiencing the same mosquito control issues, so encouraging the resident to share the information with others engages this larger audience and makes a greater impact in communities. Finally, share ideas for source modification, whether it is screening a drain or modifying a rain barrel. Working together is beneficial for everyone involved.

Results

By combining inspections and education, the resident will see results and have the tools to finish clearing their yard of mosquito sources saving the District time now and in the future as properties are more likely maintained free of standing water. This is a big time saver for properties with multiple sources and holds the resident accountable for their property. Residents, in turn, teaching others reaches a bigger audience and encourages them to be more proactive. Fewer repeat requests allows focusing on new Service Requests and decreases response time. Lifelong learning teaches the resident that pesticide applications won't fix all their problems. Brainstorming problem mitigation as a team teaches both parties and sparks creativity. It may seem that doing both education and control is more time consuming than only focusing on one or the other at a time, however, saving a little time upfront may not necessarily save time in the long run and may not be as effective.

Conclusion

Even though Service Requests increased drastically and resources are limited, the District staff were still able to stay within a 48-72 hour response time. By simultaneously inspecting and educating, areas with high call volumes experienced a decrease in vector control demand (Fig. 4). Ultimately, why limit yourself to only one, control or education, when you are able to concurrently do both.

Acknowledgements

Special acknowledgements to the following GLACVCD staff: Martin Serrano, Yessenia Curiel, Rudy Serrano, Danyelle Frakes-Lewis, Edna Chavez and Ernesto Sanchez.

Aedes Ladies Are Having a Spa Day

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INTRODUCTION

On October 4, 2018 the first *Aedes aegypti* were detected in Lancaster, CA and traced to an abandoned hot tub. Our paper describes the subsequent detection of additional populations within the arid High Desert and describes the importance of unused hot tubs as a frequently utilized larval habitat. Fig. 1 shows the different places that *Ae. aegypti* were detected in the Antelope Valley.

METHODS

The initial positive hot tub was completely disassembled and thoroughly examined for eggs. About 14 m (46 ft) of pipes were removed from the spa and cut into approx. 2.5 cm (1 in) pieces. These were inspected and sorted for the presence or absence of *Aedes* eggs. The pieces with eggs were placed into buckets and flooded with water, then were checked for larvae for several days. Larvae were collected, placed into emergence jars and reared to adults for identification. After larval activity subsided, water was drained and the pipe pieces were left to dry.

Because not all *Aedes* eggs hatch at the same time (installment hatching), this process was repeated until no additional larvae hatched. Then the pieces were washed in a 10% bleach solution, scrubbed with a brush and disposed in the trash. For the other positive sites, parts of the pipes, pool toys, and rocks were examined and eggs were collected with tape from a plant pot. These were treated using the same methods described above.

All *Aedes* detections were followed by a door-to-door campaign within a 120 m (400 ft) radius, with door hangers and letters or postcards. The sites and some surrounding properties were monitored weekly with BG Sentinel traps, and In2Care traps were set for the remainder of the season.

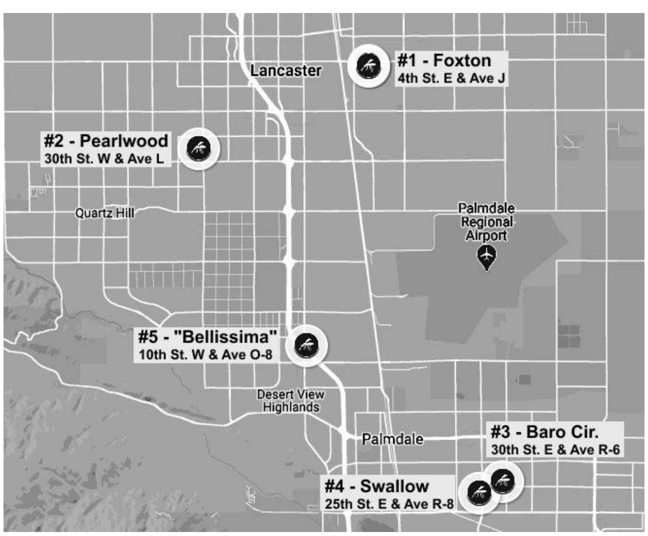

Figure 1.—Locations where *Ae. aegypti* were found in the Antelope Valley

RESULTS AND DISCUSSION

The first detection of invasive *Aedes* was in a Lancaster neighborhood (Fig. 1) and a door-2-door inspection found an unused hot tub to be the likely source of the problem. After disassembling, the total length of the pipes removed measured 14 m (46 ft). The pipes were cut into approx. 2.5 cm (1 in) pieces and a visual inspection of the pieces found a total of 4.5 m (14.7 ft) (approx. 32%) had *Aedes aegypti* eggs in them (see Fig. 2 and 3). The pieces with eggs were placed into a bucket filled with water and produced larvae for 4 flooding cycles. Additional flooding didn't hatch additional larvae.

The mechanisms for *Ae. aegypti* introduction into the second and fourth properties (Fig. 1) were inconclusive, but both had unused hot tubs on the premises that had evidence of *Ae. aegypti* infestation. The third detection was positively attributed to landscape rocks that had been brought there from an area in the San Fernando Valley that was a hotbed for *Aedes* at the time. The resident acquired free landscape rocks that had been stored in buckets where

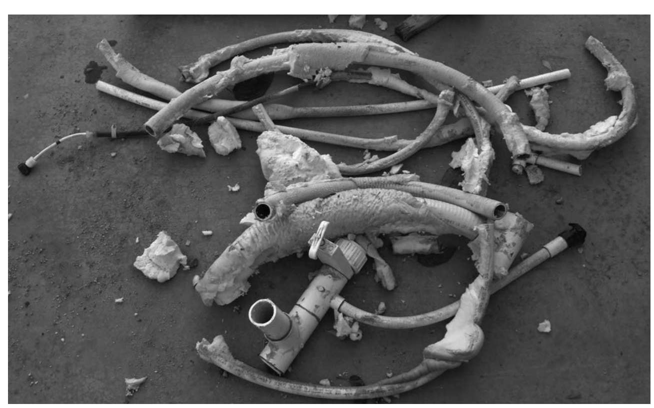

Figure 2.—Pipes removed from spa

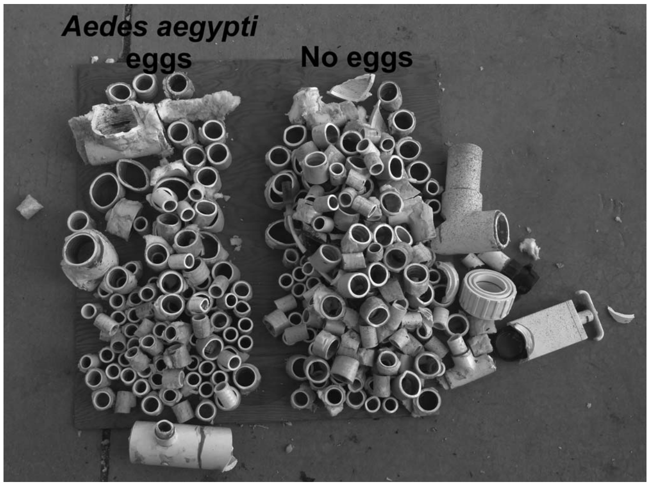

Figure 3.—Pipe pieces with *Aedes* eggs on left and without eggs on right

Figure 4.—Landscape rocks as means to transport *Aedes* into new areas]

sprinkler water hit them (Fig. 4) from a housing complex in Northridge. He used a mosquito trap to capture the *Ae. aegypti* inside his house. We inspected the buckets in his garage and the rocks, the sides of the buckets and some plastic material were covered with eggs. Greater Los Angeles County Vector Control District was notified of the find and confirmed that this was from an area with high occurrences of *Ae. aegypti*.

The fifth detection was at a nail salon that reported biting mosquitoes inside. An evaluation of the location found *Aedes* larvae inside a decorative pot that was holding a tree. The tree was an unlikely source of introduction, as it had been there for several years. Further investigation found that the bank next door had several lucky bamboo plants that may have started the infestation, although none of the employees there noticed mosquitoes.

CONCLUSION

Aedes Ladies exploit spas, including the pipes and filters of unused hot tubs, landscape rocks when they are stored in buckets of water, and nail salons with standing water in plants.

These were all unrelated introductions of the *Ae. aegypti* into the Antelope Valley. We were able to positively trace one of them to landscape rocks that were brought here from the San Fernando Valley that was a hotbed for *Ae. aegypti* at the time. It is important to look for eggs in seemingly unlikely places, such as inside of spa pipes and landscape rocks or pool toys that had been submerged in water. This emphasized the importance in messaging to include information about scrubbing containers and things brought from other areas to decrease the chances of introducing invasive *Aedes* to a new region.

ACKNOWLEDGEMENTS

We acknowledge our pool teams who were essential in the detection of *Ae. aegypti* and the follow-up outreach in those neighborhoods.

Paper Records to Live Dashboards - Combining simple forms with free data visualization tools

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Abstract

Paper records remain a small but important part of some of Sacramento-Yolo Mosquito and Vector Control District (District) operations, because it is difficult to match the speed, ease of use, and longevity of writing something down. However, as more and more data are collected, it becomes difficult to answer new questions, and much of that data goes locked away into boxes. Simple forms combined with free data visualization tools let the District unlock the data and look at it in new ways. Using the free Google forms, Sheets with pivot tables, and the new Google Data Studio, we show how a simple entry form can create a database to expand into a range of live reports, maps, charts and graphs to answer the questions about these data now and in the future.

The Discovery of *Aedes aegypti* (Diptera: Culicidae) in the City of Modesto, Stanislaus County, California and the initial Response

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INTRODUCTION

East Side Mosquito Abatement District (District) first established June 26, 1939 provides mosquito control services covering over 540 square miles with approximately 100,000 parcels of property in the northern portion of Stanislaus County. The cities served by the District include Modesto, Oakdale, Riverbank, Salida and Waterford, and the municipal communities of Empire, Knights Ferry and Valley Home, all north of the Tuolumne River. Cities located to the south of Tuolumne River in Stanislaus County are served by our sister agency, the Turlock Mosquito Abatement District.

Until July 25, 2019 when East Side Mosquito Abatement District found *Aedes aegypti* in its jurisdiction, the furthest north in California's Central Valley where it had been discovered was in the city of Merced, Merced County, two years earlier. In early June 2013, *Ae aegypti* was discovered at a residential property in the city of Madera, Madera County. Two weeks later, there was a similar discovery in the city of Clovis, Fresno County. Further discoveries were made south of these locations the same year as new localities continued to be infested at a rapid pace (Metzger et al. 2017) consistent with the spread of similar invasive *Aedes* (Montecino et al. 2014). Additional discoveries of *Ae. aegypti* were made in the cities of Menlo Park and Atherton, San Mateo County in October 2013 and in a single city in Alameda County in 2015 where it was successfully eliminated without new populations being found during the succeeding years. *Aedes aegypti* more recently was discovered in Merced County in July of 2017 (Rhiannon Jones, Personal Comm.). Additional populations of *Ae. aegypti* were discovered thereafter in 2019 in the counties of San Joaquin, Sacramento, and Placer (Boisvert et al. 2020, Huang et al. 2020). Herein, we describe activities undertaken by East Side Mosquito Abatement District since the discovery of *Ae. aegypti* in the city of Modesto that may have significantly impacted the population and potentially slowing its establishment over the coming year(s).

SEQUENCE OF EVENTS

On July 25, 2019, staff identified a male *Ae. aegypti* collected in a Reiter-Cummings Gravid trap (gravid trap) set the previous evening at a property in the Lakewood/Scenic community, on the east side of the city of Modesto, Stanislaus County, California. The mosquito was positively identified by the Seasonal Mosquito Control Technician Victor Salazar and later confirmed by the District entomologist. On July 30, 2019, a BioGent (BG) Sentinel trap placed within 150 meters of the index trap location within the same neighborhood collected a second male *Ae. aegypti*. Staff continued with more trapping in the neighborhood, conducted door-to-door inspections over the next three months, and employed several control measures to reduce the larval and adult abundance of *Ae. aegypti*. The joint press release issued by the District and Stanislaus County Public Health Agency on August 1, 2019 generated publicity in the general media market of Modesto, Stockton, and Sacramento and may have facilitated the discovery of *Ae. aegypti* in the counties of San Joaquin, Sacramento, and Placer over the following 4-6 weeks in 2019 (Boisvert et al. 2020).

To control adult mosquitoes in the neighborhood being collected in the traps, the District conducted two back-to-back applications of deltamethrin (DeltAGard®) at the rate of 6.1 ounces per acre (a.i. 1.34×10^{-4} Ibs/acre) in the affected communities. The area selected for initial treatment encompassed a half-mile radius around the index location of *Ae. aegypti* discovery and encompassed a total of 450 acres (Fig. 1). The truck-mounted applications of deltamethrin were conducted on the nights of 25 Aug 2019 between 0200 and 0500 h used ULV London Fog™ 18-20 to treat 900 acres (Fig. 1).

Additional trapping targeting *Ae. aegypti* were conducted twice a week using eight gravid traps, six BG Sentinel traps, and twelve CO_2-baited traps within the Lakewood and Scenic neighborhood of Modesto and the rest of the city of Modesto through November 15, 2019. On August 23 and August 27, 2019, single adult *Ae. aegypti* were collected in a BG Sentinel trap and a CO_2-baited trap, respectively, placed about 275 meters away from the index collection site. Nine additional adult *Ae. Aegypti,* were collected using both BioGents (BG) sentinel trap and CO_2-baited trap on September 26, 2019 at a third positive property that had multiple containers and overgrown vegetation (Fig. 2). Door-to-door inspections conducted on

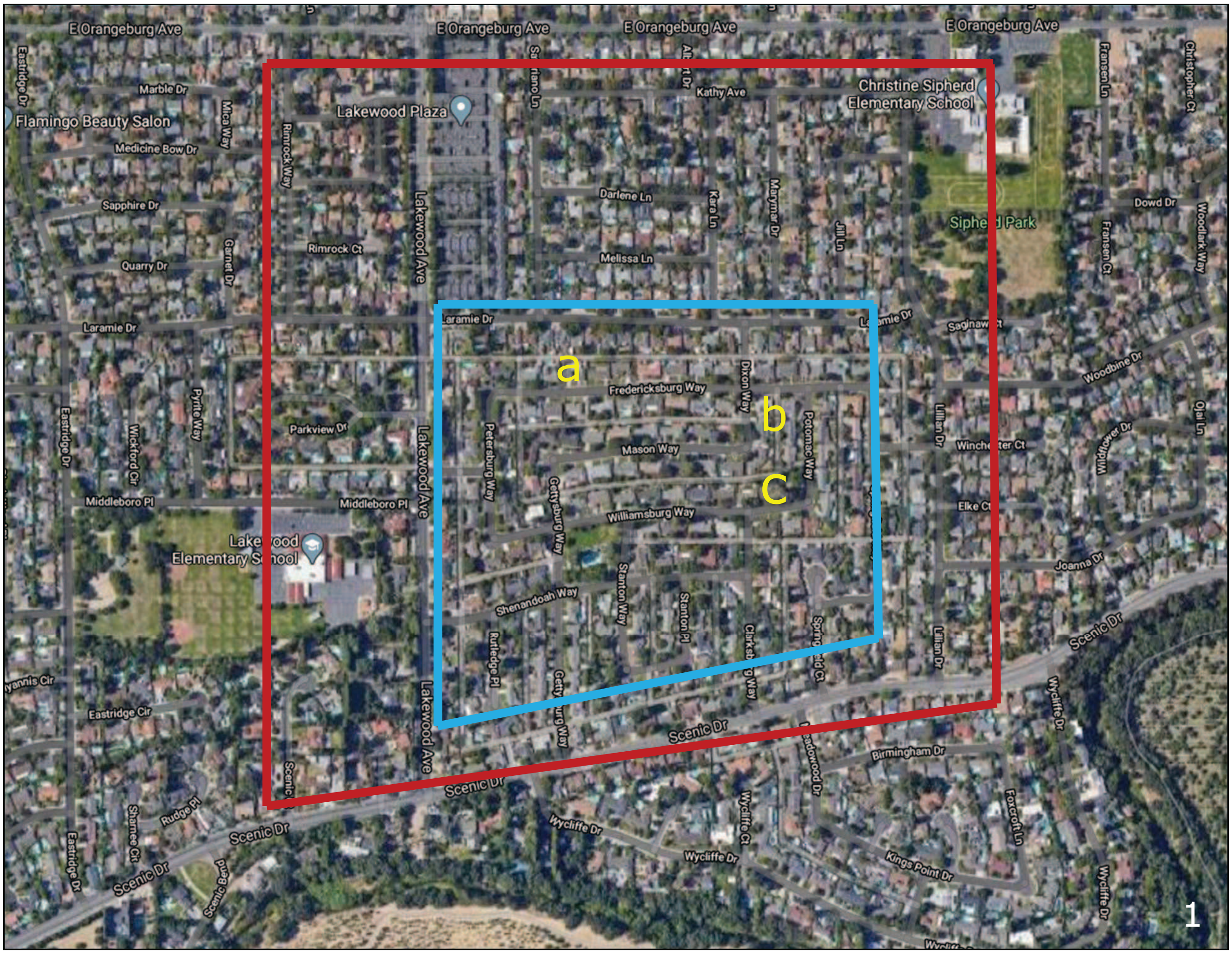

Figure 1.—The Lakewood/Scenic Community of Modesto, California where *Ae. aegypti* was first collected on 25 Jul 2019 (a). Second collection on 30 Jul2019 (b), and sixth and seventh collection with nine adult mosquitoes on 26 Sep 2019 (c). Area treated by London Fog 18-20 with adulticide (red line) area treated by A-1 Super Duty Larvicide Sprayer with VectoBac WDG (blue line).

properties within a quarter mile radius surrounding these three *Ae. aegypti*-positive properties found 13 of 44 properties with containers holding water that had the potential to produce invasive *Aedes*, although none was found. These 13 properties were treated with VectoBac® WDG (37.4 % *Bacillus thuringiensis* israelensis) using a Stihl SR 450 backpack sprayer at 0.5 Ibs/acre and a handheld Colt-4™ Fogger with Anvil 10-10 (Sumithrin/PBO) at 2.5 oz. per acre. Three of 98 containers holding water at one property were positive for invasive *Aedes* eggs.

On August 22, 2019, Wide-Area Larviciding System (WALS)™ was employed to treat approximately 105 acres within the *Ae. aegypti*-infested neighborhood surrounding the three positive properties using VectoBac WDG applied by the A-1 Super Duty Larvicide Sprayer. This application delivered VectoBac WDG at the rate of 0.5 Ibs/acre, and the treatment was repeated on August 29, 2019 for a total of 206 acres treated.

The last *Ae. aegypti*-positive trap collection was on 17 Oct 2019, and no additional invasive *Aedes* were collected from any traps set through the end of the trapping season on 15 Nov 2019. During the entire 2019 mosquito season 14 adult *Ae. aegypti* were collected from three properties within a 300-meter radius within the Lakewood Ave and Scenic Drive community of Modesto (Fig. 1). Three of 98 containers found during door-to-door inspections were positive for invasive *Aedes* mosquitoes. Trapping for the next season is planned to start on 1 Apr 2020.

CONCLUSIONS

Aedes aegypti affected our District in many ways. The district relies heavily on recurrent Seasonal Mosquito Control Technicians to supplement five full time Mosquito Control Technicians to provide mosquito control services from April 1 through October 30 every year.

Figure 2.—The property (2a) heavily vegetated in the front yard with years of (2b) accumulated containers, old cars, and assorted refuse holding standing water, some with eggs and larval stages of *Ae. aegypti* found on 26 Sep 2019.

During the 2019 find of *Ae. aegypti* in the District, no additional employees were hired to increase our services; however, current staff were partially re-assigned from their regular duties to provide *Ae. aegypti* surveillance and control. The 2019/2020 budget was amended to track the cost of controlling *Ae. aegypti* in terms of overtime and product supplies throughout the budget year. To make our best attempt in managing *Ae. aegypti*, we must consider whether our current strategy of relying heavily on seasonal staff to supplement the five full time field staff is sustainable. In addition, we should consider all strategies to help revamp our surveillance and response plan for invasive *Aedes* while managing traditional challenges of vectors and vector-borne diseases.

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First detection of *Aedes aegypti* in Placer County (Northern California)

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Aedes aegypti were first detected in the California Central Valley and the Bay area in 2013. In 2014 and 2015 detections were reported in Southern California, where they became established. In 2017, their presence was observed again in the same as well as new Central Valley counties. Questions asked included:

1) Could these mosquitoes continue to move further north in California?
2) Would they eventually become established in Northern California which lies near the 10°C January isotherm that marks the northern limit of the range of *Ae. aegypti*?

Not everyone agreed. Moving further north could be "unlikely", because of environmental and weather conditions, or possibly over time northward dispersal would continue.

The answer to the first question is 'yes'. After being "stopped" at Merced for almost two years, *Ae. aegypti* moved north during July to August 2019 and was detected in five municipalities in four different counties, including Stanislaus, San Joaquin, Placer and Sacramento. We still have to confirm if they have become established.

The Placer Mosquito and Vector Control District (the District) started to work on an Invasive Species Response Plan in 2016. During that year, a first draft of the plan was produced and the District did mock inspections in backyards to mimic a real infestation by *Ae. aegypti*. In 2017, the Board of Trustees approved the Response Plan that included four steps: 1) Detect, 2) Eradicate, 3) Monitor, and 4) Manage. The District also put in place a surveillance program at 20 locations with BG Sentinel traps, Autocidal Gravid Ovitraps (AGOs) and ovicups. Sampling results were negative until 28 Aug 2019 when the District confirmed for the first time the presence of *Ae. aegypti* (a female) in one of the weekly surveillance traps (Fay trap) for West Nile virus. The District confirmed the identification with the local California Department of Public Health (CDPH) personnel in Elk Grove, CA. At the same time, the local CDPH representative was invited to a special meeting to share experience and expertise acquired with other districts facing similar issues.

During the special meeting, topics such as operational resources (staffing), control operations (priorities), and communications (employees, people, media, health agencies, etc.) were discussed. Decisions were made based on the accepted response plan and new information provided by recent detections experienced by neighboring districts.

The first step of the response plan is to **Detect**. Here is a timeline summary of the different detections observed in Placer County:

1) First *Ae. aegypti* detected in south Placer County on 28 Aug 2019 only a few miles from the District office.
2) On 29 Aug 2019, Sacramento-Yolo Mosquito and Vector Control District announced the first detection in Sacramento County which was adjacent to our initial detection. On the same day, 20 BG Sentinel traps (with lure, but no CO_2) were deployed close to the county line between Placer and Sacramento where the first specimen was caught.
3) A second detection of *Ae. aegypti* in the same area was made on 4 Sep 2019, but this time in a BG Sentinel trap.
4) Two more detections (still in the same area) were made on 30 Oct and 1 Nov in BG Sentinel traps. There was almost a two-month gap between the second (9/4/19) and third detections (10/30/19).

The second step of the response plan is to **Eradicate**. We attempted to eradicate *Ae. aegypti* from the first detection area using four methods:

1) A ground adulticide treatment (DeltaGard) with a truck mounted fogger was performed before sunrise on 29 Aug and on two other dates (31 Aug and 1 Sep).
2) Door-to-door inspections were done from 29 Aug until 13 Sep 2019.
3) Five Wide Area Larvicide Spray (WALS) treatments were performed using VectoBac WDG. Sacramento-Yolo MVCD performed all five treatments with their A-1 turbine sprayer. Figure 1 shows the equipment used for the WALS spraying.
4) From 29 Aug to 13 Sep additional treatments included adulticide (Zenivex E4) using handheld foggers and larvicide (Natular DT tablets) treatments in backyard drains, rain barrels and other containers.

While the District employees inspected properties, they were asked to document the different sources that could contain water and potentially support the development of *Ae. aegypti*. Fifteen types of sources were identified during the survey (Fig. 2). A comparison between two different streets within the area of concern showed similar results

Figure 1.—Picture showing the A-1 turbine and 100 gallon tank on a flatbed truck. This equipment was used for the larviciding treatments using the VectoBac WDG.

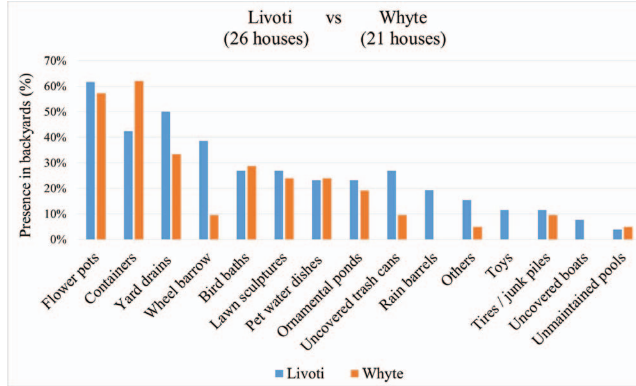

Figure 2.—Percentages of different sources found on properties during inspections. Results compare properties visited on two different streets of the infested area in Placer County.

(Fig. 2). Flowerpots, containers, and yard drains were present in about 50% of the 150 properties inspected. Based on that experience, the District is proposing a research project for the summer 2020 that will consist of inspecting properties in five different municipalities where *Ae. aegypti* could potentially be found. This project will have multiple goals:

- Continue to document different containers where *Ae. aegypti* could potentially develop in Placer County
- Determine if the ratios of the various sources/containers differ among environment types/municipalities.
- Train our employees to perform inspections
- Create an internship for a student to participate in this research project and develop expertise and gain science experience

The detection of *Ae. aegypti* in Aug 2019 had many ramifications. Because the District wanted to provide the most accurate and timely information to the public, the website and social media were updated as needed, and email notifications were sent when treatments (WALS larviciding and large scale ground adulticiding using trucks) were scheduled. New outreach materials including door hangers, fact sheets, news releases, etc. were created to respond to the new threat. The District's General Manager and the Public Information Officer responded to media (digital/broadcast and print, radio, etc.) requests. Because the presence of *Ae. aegypti* was detected concurrently in Sacramento County, requests from the media were "shared" by both counties.

Although sometimes the message from the media was focused more on the disease aspect of the presence of *Ae. aegypti*, the District decided to address this new presence with relevant talking points that corresponded to the District's three "Ds":

- **Drain** or **dump** any standing water that may produce mosquitoes and permanently remove water-holding containers. This message was particularly relevant to the presence of *Ae. aegypti,* because this species thrives in a backyard environment where many potential sources (see Fig. 2) can be found.
- **Defend** yourself and your home by using a CDC-recommended insect repellent and dressing protectively when outside. Make sure screens on doors and windows are in good condition.
- Contact the **District** for any additional help controlling mosquitoes around your home.

The third step of the response plan is to **Monitor.**

As a first step in the monitoring phase, the District wanted to determine the success of eradication efforts. Unfortunately, although the District used an "eradication" approach, the detection of only four adult *Ae. aegypti* over a two-month period did not provide an indication of eradication success. District technicians were available to respond to new detections as needed and a surveillance program with BG Sentinel traps was implemented after the first detection and remains on-going during the winter 2020.

The fourth step of the response plan is to **Manage.**

At the District, operational priorities are established every year. For the last years, West Nile virus always has been the top priority based on the real health threat to Placer County's population. Although the presence of any invasive species (*Ae. aegypti* or *Aedes albopictus)* was never detected before 2019, conducting monitoring and management for those species was the next priority after West Nile virus.

To prepare for their eventual detection, an emergency fund for an invasive mosquito response was established by the District in 2015 to cover 24 months. The target was to accrue $700,000 that would cover operations such as:

- Surveillance $100,000
- Control $300,000
- PR/Outreach $150,000
- Logistics/IT $150,000

A balance close to $500,000 was available by Aug 2019 when the first *Ae. aegypti* was detected.

In 2019, the District spent around $45,000 for surveillance, control and public outreach expenses. Expenditures are anticipated for the 2020 invasive mosquito response. Although the extent of resurgence or detection of additional infested areas is not known at this time, the District is planning to secure funds for additional larvicide and adulticide products, increase public outreach (raise awareness and acceptance by the public), hire more seasonal employees (up to ten compared to five in 2019), and hire two new full-time technicians. More employees also require more vehicles to conduct operations in the field.

Although the District still has emergency funds, it will need additional funds to sustain its operations at a level where it can guarantee the proper safety of Placer County residents. If repeated infestations or an endemic population becomes established, additional long-term operational funding will be required.

What is the District's plan? First, the District would like to engage the public to gain its support for a long-term operational response. Placer County's population is familiar with the District's response plan and approach relative to West Nile virus. The public is not familiar with the new threat of invasive species, how their presence will degrade the outdoor residential environment, and how the District is planning to address that threat. The District also needs to identify a core *Aedes* task force and a budget for District staff and operations.

Once the extra operations budget needed to extend beyond 2021 is identified, the District will need to design a funding proposal consisting of either a special tax or new benefit assessment to generate sufficient funds to sustain these new invasive *Aedes* activities. Initiating a ballot process is an expensive and lengthy process. New money will be needed if the District does not want to deplete emergency funds from 2015. With only four adult *Ae. aegypti* caught so far and no proof of an established population, the District needs to find the right approach and timing to educate and engage the public to contribute and approve a benefit assessment.

Conclusions: The detection of *Ae. aegypti* in August 2019 was 'game changer' for the District requiring major changes to address new compared to previous operations. The District learned from our fall 2019 approach and our response to contain this new species. Based on this experience combined with the response of other districts who faced a similar problem in 2019, the District is preparing for 2020.

A deep investigation into the virome of *Culex* mosquitoes in California using small RNA sequencing

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Introduction

One of the greatest global health threats posed by mosquitoes comes from the transmission of arboviruses such as West Nile Virus (WNV). There is evidence that the ability of the *Culex* mosquito vector to transmit these viruses depends to some extent on bacteria and other viruses present in the vector, such as insect-specific viruses (ISVs), yet we have an incomplete understanding of the viruses infecting *Culex* mosquitoes from the field and how the mosquito immune system responds to these infections. Herein, we aim to elucidate the virome, including both currently known and unknown viruses, of *Culex* mosquitoes in California as well as characterize the small RNA immune responses generated by these mosquitoes to particular viruses. Such information will be crucial in determining how these viruses may impact the ability of vectors to transmit arboviruses.

Methods

The extraction and purification of small RNAs for deep sequencing were extracted using Illumina platforms. Bioinformatics tools were used to process the reads and assemble contigs to catalog the virome of the ~ 2,500 mosquitoes (65 pools) that were analyzed. We used in-house scripts to further determine the type and size of small RNAs generated by the mosquito against each virus to better understand the immune response generated by each of the identified mosquitoes. Finally, we used MEGA (Molecular Evolutionary Genetics Analysis) tools to perform phylogenetic analysis to reveal potential novel viruses or divergent sequences.

Results and Discussion

Taken together, our results demonstrated the power of using small RNA sequencing to characterize the virome of Culex mosquitoes in California. We also confirmed the sensitivity of our method by detecting WNV in nearly all samples that had tested positive for WNV by quantitative PCR. Furthermore, we identified several viruses that have been previously detected in *Culex* in China and Western Australia, suggesting that infection by these viruses may in fact be more ubiquitous than previously thought and that much of the *Culex* virome may be conserved regardless of geographic location. Most importantly we detected that a majority of the viruses identified elicited an siRNA response from the *Culex* mosquitoes while a restricted number elicited a piRNA response (e.g. bunya-like virus and phasma-like virus, which are related to each other as members of the same order with negative-single-stranded RNA genomes) suggesting that properties of individual viruses influence the type of small RNA response generated by the mosquito in response to infection.

Conclusions

Our methodology can be used to detect, with high sensitivity, both known and unknown viruses in mosquito samples without any specific enrichment for viral particles. Furthermore, our findings indicate that properties of individual viruses contribute to the type of small RNA immune response generated by the mosquito, especially the activation of the less common piRNA pathway.

Acknowledgements

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A validated triplex RT-qPCR protocol to detect chikungunya, dengue and Zika viruses in mosquitoes

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Abstract

Recently, the incidence of chikungunya, dengue and Zika disease has increased due to globalization and urbanization. It is vital that reliable detection tools become available to assess viral prevalence within mosquito populations. Based on previous publications on clinical diagnosis of human infections, we describe a customized triplex RT-qPCR protocol for simultaneous detection of chikungunya virus (CHIKV), dengue virus serotypes 1-4 (DENV1-4) and Zika virus (ZIKV) in mosquitoes. In preliminary assessment to determine the specificity and sensitivity of primers and probes, all six targets were detected individually with the following thresholds as indicated by calculated plaque forming unit (pfu) equivalents: $3.96x10^{0}$ in CHIKV, $3.80x10^{1}$ in DENV1, $3.20x10^{1}$ in DENV2, $8.00x10^{-1}$ in DENV3, $1.58x10^{0}$ in DENV4, and $6.20x10^{0}$ in ZIKV. When tested in a full combination of six targets (CDZ mix), CHIKV, DENV1-4 mix or ZIKV were detected with the thresholds of $1.32x10^{0}$ in CHIKV, $3.79x10^{0}$ in DENV1-4 and $2.06x10^{0}$ in ZIKV. All targets, individually or in full combination, were detected when mixedwith *Aedes aegypti* (L.) homogenate. A robust evaluation with three replicates in each of three plates for each target of CHIKV, DENV1-4 and ZIKV individually or in full combination was conducted. In individual assays, CHIKV was detected to $3.96x10^{-1}$, DENV1-4 to $1.14x10^{0}$ and ZIKV to $3.20x10^{0}$. In full combination assays, CHIKV was detected to $1.32x10^{-1}$, DENV1-4 to $3.79x10^{1}$ and ZIKV to $1.07x10^{0}$. This triplex RT-qPCR protocol appears to consistently detect all six targets and does not cross react with *Ae. aegypti* homogenate, making it a practical assay for use among vector control agencies . These procedures were used to test *Ae. aegypti* and *Ae. albopictus* collected from the field for CHIKV, DENVs and ZIKV with negative findings.

Rattus rattus and its Association with Plants in Alameda County

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Abstract

Alameda County is the 7th most populous county in California and historically has experienced established populations of both roof rats (*Rattus rattus)* and Norway rats (*Rattus norvegicus*). Roof rats, an introduced species, are well adapted to the Alameda County landscape due to the availability of food, water, and shelter. From 2014 to 2018, roof rat requests for service received by the Alameda County Vector Control Services District have nearly tripled. Roof rats utilize various habitats including rocks, wood piles, trees, residential and commercial buildings, and dense vegetation. They pose a public health risk by harboring pathogens in their waste, contaminating food, biting people, or introducing pathogens indirectly by the ectoparasites they carry. This poster examines the plant species that provide harborage, food sources, and roof access that allow roof rats to thrive in Alameda County.

Comparative morphology of *Borrrelia burgdorferi* sensu lato and *Borrelia miyamotoi* morphology by microscopy

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Abstract

Borrelia burgdorferi sensu lato, which includes the agent of Lyme disease (*B.burgdorferi* sensu stricto) and *Borrelia miyamotoi*, the agent of a type of tick-borne relapsing fever, have been detected in the western blacklegged tick, *Ixodes pacificus*. The California Department of Public Health, Vector-Borne Disease Section individually tests *I.pacificus* by direct fluorescent antibody assay to visually screen for *Borrelia* spirochetes. However, further molecular testing is needed to identify *Borrelia* at the species level. In this poster, we analyzed if visual morphology of the spirochetes can distinguish between *B. burgdorferi* sl and *B. miyamotoi*.

Dirofilaria immitis prevalence in Southern California's invasive *Aedes* and native *Culiseta* species

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Abstract

Vector-borne diseases, especially those transmitted by mosquitoes, are of growing concern to public health and vector control centers. Increased disease reports are commonly attributed to an increase in the local vector population. Since 2013, Dog Heartworm Disease cases have increased by approximately 21% in the United States as reported by the American Heartworm Society. During the same time, invasive mosquitoes belonging to the genus *Aedes* have been found in Southern California. *Aedes aegypti, Aedes notoscriptus* and *Aedes albopictus* continue to spread across North America and especially along the California coast. The purpose of our research is to determine the prevalence of *Dirofilaria immitis,* the filaria causing Dog Heartworm, within Southern California invasive mosquitoes. A native California species, *Culiseta incidens*, also was tested to determine filarial prevalence.
The Greater Los Angeles County Vector Control District (GLACVCD) collected *Aedes* mosquitoes to be sent to the University of California at Davis for viral testing. Negative pools then were sent to the Thiemann laboratory at the University of the Pacific. Due to prior viral testing, pools were already homogenized. DNA was extracted and tested via a polymerase chain reaction (PCR) to determine the presence of *D. immitis* using a *D. immitis* specific primer, 5s. *Culiseta incidens* mosquitoes also were collected by GLACVCD and then were dissected in the Thiemann laboratory. Head and thoraces were separated from the abdomen to determine the prevalence of an infective stage L3 filaria only found in the head and proboscis region of the mosquito. DNA homogenization and extraction were preformed prior to PCR testing using two primers, one specific to *D. immitis*, while the second panfilarial primer recognizes any type of filaria present. Determining the prevalence of other filaria is important in understanding the ability of these mosquitoes to transmit various filarial worms. Twenty percent (20%) of the total positive pools were sent for sequencing to confirm the presence of *Dirofilaria immitis*.

Diversity and distribution of Borreliae in San Mateo County, CA 2018-2019

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Abstract

The *Borrelia burgdorferi sensu lato* (*Bbsl*) complex contains both pathogenic species (such as *Borrelia burgdorferi sensu stricto*, the causative agent of Lyme borreliosis), and nonpathogenic species. We sequenced *Bbsl* positive tick pools collected by the San Mateo County Mosquito and Vector Control District using a conventional, nested PCR assay targeting the rrf-rrl region to determine the prevalence of pathogenic *Borreliae*. Of 102 total *Borrelia*-positive tick pools, 49 were identified as *B. miyamotoi* and 53 were identified as *Bbsl*. Sequencing of the *Bbsl* isolates revealed 45.28% (n=24) were *B. burgdorferi sensu stricto*; 28.3% (n=15) were *Borrelia bissettiae*; and 26.41% (n=14) could not be sequenced. As local residents rely on surveillance information to make effective decisions about tick-borne disease prevention, additional information about coastal California *Bbsl* ecology is necessary to mitigate disease risk, increase awareness, and dispel misconceptions.

Introduction

Lyme borreliosis is a multisystem illness caused by the pathogen *Borrelia burgdorferi sensu stricto (Bbss)* and is the most commonly reported vector-borne disease in Europe and the United States (Burgdorfer et al. 1982, Murphree Bacon et al. 2008). *Bbss* is transmitted by the bite of infected hard-bodied ticks belonging to the *Ixodes ricinus* complex. In California, the Lyme disease vector is the western black-legged tick (*Ixodes pacificus),* which people often encounter during recreational use of the outdoors, rather than exposure near the home environment (Salkeld et al. 2019). Because California has a strong outdoor recreational community, surveillance is necessary to convey accurate information to the public so that appropriate personal protective measures can be taken.

The San Mateo County Mosquito and Vector Control District (SMCMVCD) conducts yearly surveillance for ticks and tick-borne pathogens in San Mateo County, CA. *Ixodes pacificus* are collected by flagging from trails in parks and natural preserves, and then tested using qPCR assays for several tick-borne pathogens including *Borrelia miyamotoi* and *B. burgdorferi* (Barbour et al. 2009). Problematically, *B. burgdorferi* is part of a larger complex of 18 different spirochete bacteria that can be evenly divided into potentially pathogenic (characterized by being reported or isolated from humans) and non-pathogenic subgroups. The qPCR assay used to detect *Borrelia spp*., cannot differentiate among these subtypes and thus cannot identify which isolates are pathogenic. For this reason, surveillance results are reported as *B. burgdorferi sensu lato* (*Bbsl*) and the minimum infection prevalence (MIP) values for ticks do not indicate the actual risk of contracting Lyme borreliosis.

The urbanized San Francisco Bay Area has a diversity of both pathogenic and nonpathogenic *Borreliae*. A study conducted in Alameda County in 2014 identified six *Borrelia* variants, including pathogenic *B. burgdorferi sensu stricto (Bbss)* and *B. bissettiae* (Rudenko et al. 2011); non-pathogenic *B. americana* and *B. californiensis*; and two newly described *Borrelia* species (Fedorova et al. 2014). Although both *Bbss* and *B. bissettiae* utilize the same vectors, they inhabit slightly different ecological niches. *Bbss* is reservoired predominantly by sciurids (Salkeld et al. 2008), whereas *B. bissettiae* is reservoired predominantly by woodrats (*Neotoma* spp.) and associated murine species (*Peromyscus* spp.) (Brown and Lane 1992). The lack of understanding of the pathogenicity of these novel variants and the unknown distribution of pathogenic species, makes genotyping *Borrelia* a critical step in tick-borne disease surveillance. Herein, we sequenced historical DNA isolates from the SMCMVCD archive to identify *Borreliae* variants present in San Mateo County and to determine their distribution.

Materials and Methods

Adult and nymphal *Ix. pacificus* ticks were collected from 24 and 13 recreational areas in San Mateo County, respectively, in 2018–2019 (Figure 1 and Table 1). Collection of both adults and nymphs utilized a 1-m square piece of white flannel cloth attached to a wooden dowel that was dragged through tall grass at the side of trails. Our goal was to collect 200 ticks per park on a maximum of

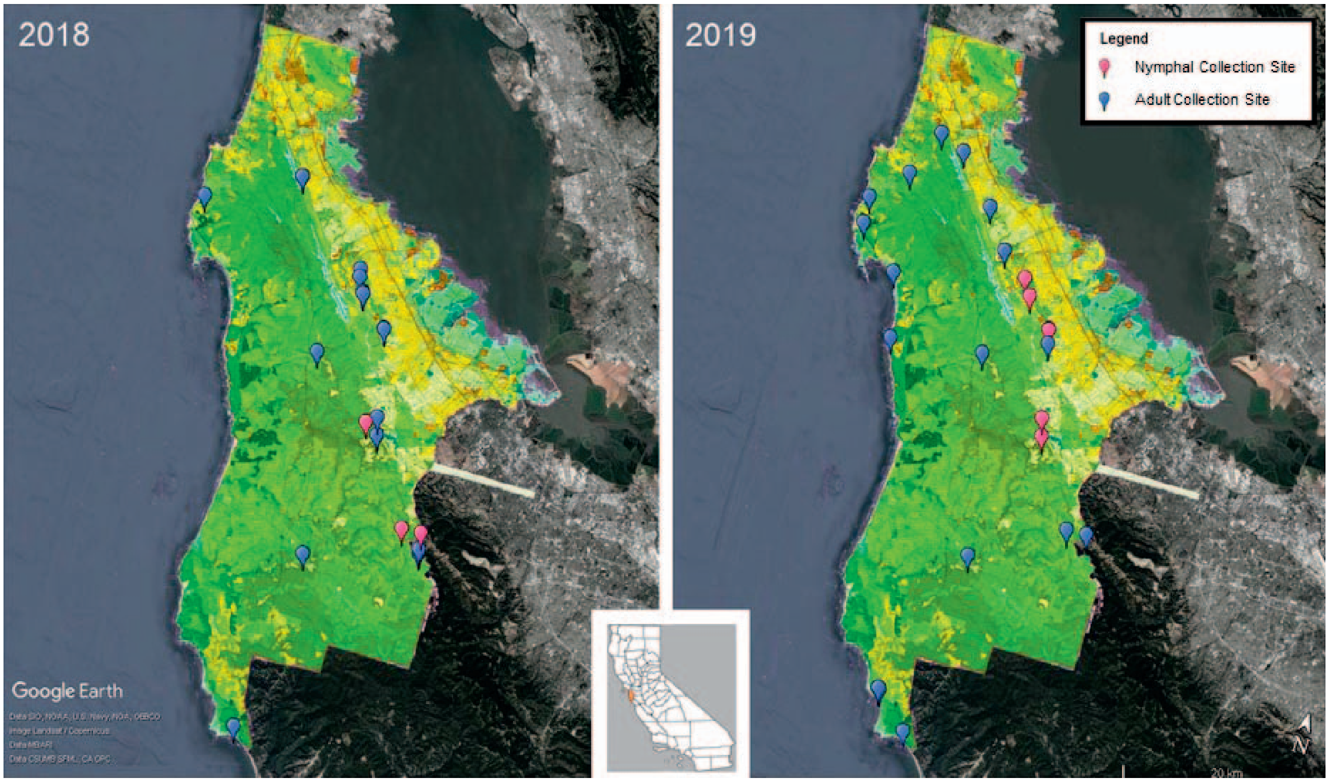

Figure 1.—Location of *Ixodes pacificus* tick collections from San Mateo County, CA, 2018–2019

Table 1.—List of collection locations for *Ixodes pacificus* ticks collected in 2018-2019 from San Mateo County, CA with notes on habitat type, tick abundance, and location with respect to the California Coast Range.

Park Name	Region	Habitat Type	Dominant Vegetation	*High* Risk
Ano Nuevo State Park	Pacific Coast	Coast grassland	Grassland/Herbaceous shrub	**yes**
Big Canyon Park	East Coast Range	Hardwood Forest	Oak Woodland	**yes**
Coal Creek OSP	Central Coast Range	Hardwood Forest	Oak Woodland/Grassland	**yes**
Costanoa Recreational Area	Pacific Coast	Coast grassland	Coastal Scrub/Riparian	**yes**
Crystal Springs Reservoir	East Coast Range	Riparian	Oak/Woodland	**yes**
Eaton Park	East Coast Range	Urban Adjacent	Oak Woodland	**yes**
Edgewood Park	East Coast Range	Hardwood Forest	Oak Woodland	**yes**
Frontierland	East Coast Range	Urban Adjacent	Oak/Coyote brush	no
Half Moon Bay Coastal Trail	Pacific Coast	Coast grassland	Grassland	no
Hillsborough OS	East Coast Range	Urban Adjacent	Oak Grassland	**yes**
Junipero Serra Park	Central Coast Range	Mixed Hardwood/Evergreen	Oak Woodland	no
Laurelwood Park	East Coast Range	Urban Adjacent	Oak/Woodland	**yes**
Los Trancos OSP	Central Coast Range	Hardwood Forest	Oak/Woodland/Grassland	**yes**
Montara Mountain	\Vest Coast Range	Coast grassland	Grassland/Herbaceous shrub	**yes**
Memorial Park	Central Coast Range	Evergreen Forest	Redwoods	no
Purisiwa Creek OSP	Central Coast Range	Evergreen Forest	Redwoods	**yes**
Rancho Corral de Tierra (GGNRA)	West Coast Range	Coast grassland	Grassland/Herbaceous shrub	no
San Pedro Valley OSP	West Coast Range	Herbaceous Grassland	Coyote brush	**yes**
Skyline Ridge OSP	Central Coast Range	Hardwood Forest	Oak Woodland/Grassland	**yes**
Sweeney Ridge (GGNRA)	West Coast Range	Coast grassland	Grassland/Herbaceous shrub	no
Thomewood OSP	East Coast Range	Hardwood Forest	Oak Woodland	**yes**
Water Dog Lake Park	East Coast Range	Urban Adjacent	Oak Woodland	**yes**
Wavecrest Open Space	Pacific Coast	Coast grassland	Grassland	no
Wunderlich OSP	East Coast Range	Hardwood Forest	Oak/Woodland	**yes**

Definitions: GGNRA {Golden Gate National Recreation Area); OSP {Open Space Preserve) three occasions. Ticks were brought back to the laboratory and kept at 4 °C until they could be identified to species using a California specific dichotomous key (Furman and Loomis 1984). Ticks were separated by collection, species, sex, and stage and pooled in groups of five adults or two nymphs. Pools were placed in reinforced 2-ml tubes with five ceramic beads and 700µl of Lysis Binding Solution Concentrate (Applied Biosystems) diluted with 100% isopropanol. Tubes were placed in an Omni Bead Ruptor (OMNI International) tissue homogenizer and physically disrupted for 30 sec. DNA was purified using the MagMax Viral RNA Isolation Kit (Applied Biosystems) according to the manufacturer's recommendations. A multiplex, qPCR assay for *B. miyamotoi* and *Bbsl* (Barbour et al. 2009) was conducted, and pools that were positive by qPCR for Bbsl were subject to *Borrelia* genotyping.

Nested *Borrelia burgdorferi* PCR Assay

Borrelia burgdorferi sensu lato variants were identified using a nested PCR assay that targets the variable spacer region between the 3′ end of the 5S rRNA (rrf) and the 5′ end of the 23S rRNA (rrl), with primers and run protocol as described by Barbour and Cook (2018). Samples were run in triplicate with a total of six negative controls and a single positive control. PCR products were checked via gel electrophoresis and positive samples with a band size of 200-bp were purified using Agencourt Ampure XP for PCR Purification (Beckman Coulter Life Sciences). Cleaned PCR product was sent to ELIM Biopharmaceuticals (Hayward, CA) for sequencing, aligned using the program GENEious, and variant identity determined by comparison with sequences from isolates in the National Center for Biotechnology Information Database (NCBI).

Statistical Analysis

A grand total of 6,898 *Ix. pacificus* ticks were collected over the two-year period (1,742 pools). In 2018 the MIP for adults and nymphs for *Bbsl* was 0.67% and 1.65% respectively and for 2019 was 0.47% and 1.32% respectively. A T-test did not detect a significant difference in MIP between years (95% conf. = -0.44, 0.65; p = 0.57) or stage (95% conf.= -1.4, 0.44; p = 0.19) so results were combined by location for Figure 1 for illustrative purposes.

Parks that had >100 ticks collected in three trips were classified as "high risk" and parks where <100 ticks were collected were classified as "low risk" (Table 1). A one-way ANOVA with samples separated by year and stage was conducted to determine if there was an effect of dominant vegetation type, region and habitat. Isolates from *Ix. spinipalpus* were not included in these analyses, because it is biologically plausible they have a different ecology than *Ix. pacificus* and including them could be confounding.

Results

Sequencing success was lower than anticipated and declined by year (83% of adult and 22% of nymphal pools from 2018 were successfully sequenced, compared to 100% of adult and 66% of nymphal pools from 2019). CT values for both *Bbsl* and *B. miyamotoi* were generally high (average CT 37 approaching the normal cut-off at CT 40) for the qPCR duplex assay which may be due to the use of an automated nucleic acid purification kit that is optimized for RNA extraction but advertised for use with all nucleic acid samples (Crews 2019).

Out of a total of 53 *Bbsl* isolates, 45.3% (n=24) were *Bbss*; 28.3% (n=15) were *B. bissettiae*; and 26.4% (n=14) could not be identified (Table 2). Out of the 102 total *Borreliae* samples obtained, 48.0% (n=49) were identified by qPCR as *B. miyamotoi*.

The one-way ANOVA detected a significant effect of dominant vegetation type on the MIP of *B. miyamotoi* ($P<0.01$). A simple linear regression model was run on the collected adults and vegetation types, which showed a significant positive effect for Grass/Shrub habitat ($p=0.03$) and Oak/Shrub habitat ($P<0.01$) on *B. miyamotoi* MIP. There was no significant effect of vegetation type, habitat or region on *Bbsl*, *Bbss*, or *Bbis* MIP. However, these results should be considered preliminary, because the number of ticks collected from each vegetation type varied significantly ($X^2=1731.3$, $P<0.01$) as did the number of ticks testing positive. Additionally, the low MIP of both *Bbsl* and *B. miyamotoi* indicated that greater sample sizes will be necessary to determine if any relationship exists between *Borrelia* subtype and habitat.

Discussion

The widespread misunderstanding of Lyme disease ecology in California highlights the need for further research into west coast tick-borne disease ecology. Approximately 67% of San Mateo County is climactically suitable for *Ix. pacificus* (Eisen et al. 2018) and the broad interface between open space and intensely developed areas creates a high risk of exposure to ticks. Delineating the distribution of the local *Borrelia* variants is an important step to providing metrics of risk for acquiring tick-borne pathogens.

We found that the *Bbsl* MIP averaged less than 1.0% in adults and less than 2.0% in nymphal *Ix. pacificus* county-wide. Of the 54 *Bbsl* positive tick pools that were successfully sequenced, only two variants, *B. bissettiae* and *Bbss,* were detected. It is possible that the duplex qPCR assay may not detect all variants. However, a retest of a 96-well plate containing 394 ticks (84 pools) using the rrf-rrl assay described above did not detect additional positive pools. Sampling bias may also explain the lack of variants. "High risk" parks were sampled more often than "low risk" parks and flagging is a selective method for collecting questing tick species. "Low risk" parks are often dominated by low grass or herbaceous scrub and high solar exposure which is inhospitable to questing ticks (Lindström and Jaenson 2003) but may support a variety of nest-associated nidicolous ticks with unique *Borrelia* variants.

Table 2.—Prevalence of *Borrelia miyamotoi* and *Borrelia burgdorferi sensu lato* isolates from adult and nymphal *Ixodes pacificus* ticks collected from parks and open space areas in San Mateo County, 2018-2019

Park Name	Stage	Total	# Bm (MIP)	# Bbsl (MIP)	# Bbss (MIP)	# Bbis (MIP)	Unchar.
Año Nuevo State Park	Adult	503	1 (14%)	6 (86%)	2 (33%)	4 (67%)	0
Año Nuevo State Park	Nymph	3	0	0	0	0	0
Big Canyon Park	Adult	200	0	0	0	0	0
Big Canyon Park	Nymph	7	0	0	0	0	0
Coal Creek OSP	Adult	592	5 (62%)	3 (38%)	3 (100%)	0	0
Coal Creek OSP	Nymph	81	1 (50%)	1 (50%)	0	0	1 (100%)
Costanoa Recreational Area	Adult	186	1 (50%)	1 (50%)	0	1 (100%)	0
Costanoa Recreational Area	Nymph	8	0	0	0	0	0
Crystal Springs Regional Trail	Adult	245	2 (100%)	0	0	0	0
Crystal Springs Regional Trail	Nymph	72	0	1 (100%)	0	1 (100%)	0
Eaton Park	Adult	393	2 (100%)	0	0	0	0
Eaton Park	Nymph	58	0	1 (100%)	0	1 (100%)	0
Edgewood County Park	Nymph	102	1 (50%)	1 (50%)	0	0	1 (100%)
Frontierland Park	Adult	6	1 (100%)	0	0	0	0
Half Moon Bay Coastal Trail	Adult	1	0	0	0	0	0
Hillsborough (North)	Adult	85	0	1 (100%)	1 (100%)	0	0
Hillsborough (South)	Adult	159	2 (50%)	2 (50%)	1 (50%)	1 (50%)	0
Junipero Serra Park	Adult	46	0	0	0	0	0
Laurelwood Park	Adult	304	1 (25%)	3 (75%)	2 (67%)	1 (33%)	0
Laurelwood Park	Nymph	153	1 (25%)	3 (75%)	0	1 (33%)	2 (67%)
Los Trancos OSP	Adult	421	7 (70%)	3 (30%)	3 (100%)	0	0
Los Trancos OSP	Nymph	66	0	4 (100%)	0	0	4 (100%)
Memorial Park	Adult	189	0	3 (100%)	1 (33%)	1 (33%)	1 (33%)
Montara Mountain	Adult	364	2 (100%)	0	0	0	0
Purisima Creek Redwoods OSP	Adult	477	3 (50%)	3 (50%	2 (66%)	0	1 (33%)
Rancho Corral De Tierra (GGNRA)	Adult	43	0	0	0	0	0
San Pedro Valley Park	Adult	449	5 (83%)	1 (17%)	0	1 (100%)	0
Skyline Ridge OSP	Adult	476	3 (43%)	4 (57%)	2 (50%)	0	2 (50%)
Skyline Ridge OSP	Nymph	74	2 (100%)	0	0	0	0
Sweeney Ridge (GGNRA)	Adult	15	0	0	0	0	0
Thornewood OSP	Adult	211	3 (43%)	4 (57%)	4 (100%)	0	0
Thornewood OSP	Nymph	154	3 (75%)	1 (25%)	1 (100%)	0	0
Water Dog Lake Park	Adult	293	1 (50%)	1 (50%)	0	1 (100%)	0
Water Dog Lake Park	Nymph	63	0	3 (100%)	2 (67%)	0	1 (33%)
Wavecrest Open Space	Adult	9	0	0	0	0	0
Wunderlich County Park	Adult	241	2 (100%)	0	0	0	0
Wunderlich County Park	Nymph	149	0	0	0	0	0

Definitions: Bm (Borrelia miyamotoi); Bbsl (Borrelia burgdorferi sensu lato), Bbss (Borrelia burgdorferi sensu stricto); Bbis (Borrelia bissetiae); Unchar. (Uncharacterized isolate); OSP (Open Space Preserve); GGNRA (Golden Gate National Recreation Area)

Nidicolous ticks are rarely collected through flagging and thus would not be sampled using our methods.

San Mateo County is located on the southern half of the heavily urbanized San Francisco Peninsula. It is possible that human activity and limited immigration opportunities may create an environment that supports a relatively limited and homogeneous distribution of host species. Refractory species such as western fence (*Sceloporus occidentalis*) and alligator lizards (*Elgaria multicarinata*) (Rose 1976), whose blood contains neutralizing complement proteins (Kuo et al. 2000), are present in nearly all high risk parks. Ground foraging or nesting birds may host and transport *Ix. pacificus* ticks (Dingler et al. 2014), but the majority of these birds have shown only reduced or transient host competency (Comstedt et al. 2006). Although sciurid species such as chipmunks (*Tamias* spp.) and western grey squirrels (*Sciurus griseus*) are highly competent hosts (Lane et al. 2005, Stephenson and Foley 2016), they are rare in the county, and are often displaced by eastern greys (*Sciurus carolinensis*) and fox squirrels (*S. niger*) which are infrequently infected (Salkeld et al. 2008). All of these factors likely contribute to the relatively low prevalence as well as the limited diversity of *Borrelia* variants.

The most abundant *Bbsl* and *B. miyamotoi* competent hosts found in the region are the dusky-footed woodrat (*Neotoma fuscipes*) and its associated murine species (*Peromyscus spp*.) (Eisen et al. 2004, Salkeld et al. 2018). Woodrats have been reported to have an infection prevalence of 85.7% of *B. bissettiae* in some areas (Brown and Lane 1996, Brown et al. 2006). However, their contact with *Ix. pacificus* may be somewhat limited because; a.) they are nocturnal and prefer arboreal travel routes (Linsdale 1951) whereas *Ix. pacificus* subadults are largely diurnal and rest in leaf litter (Lane et al. 1995), and b.) subadult *Ix. pacificus* strongly prefer to feed on refractory lizards (Slowik and Lane 2009). It's possible

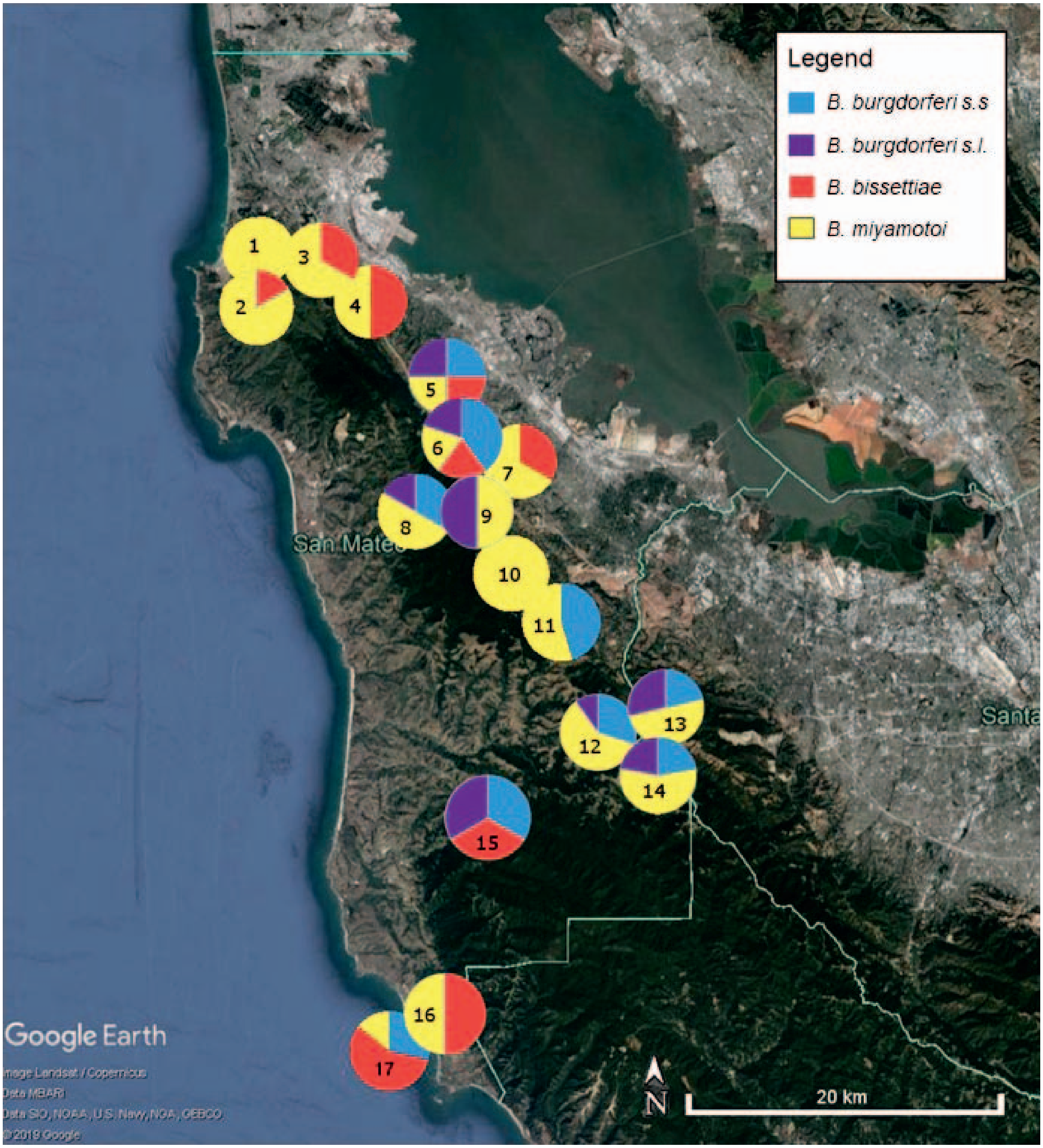

Figure 2.—Distribution of *Borrelia spp*. variants extracted from flagged *Ixodes pacificus* ticks in San Mateo County, CA, 2018-2019

that the prevalence of *Borrelia* in woodrats is supported more by nest-dwelling species of ticks such as *Ix. spinipalpus* which are competent vectors (Dolan et al. 1997) and frequently co-infest with *Ix. pacificus* (Scott 2012). Additional research on *Borreliae* cycles in hosts should be conducted in San Mateo County in order to determine if the host/nest parasite community is strongly influencing the prevalence of human pathogenic strains in questing *Ix. pacificus* ticks.

Although the low incidence of *Bbss* in San Mateo County makes it difficult to examine variation in distribution, the region can be compared to other regions to elucidate the ecology of *Bbss* and the factors that contribute to its prevalence. As local residents rely on surveillance information to make effective decisions about tick borne disease prevention, additional information about West Coast *Bbsl* ecology is necessary to dispel misconceptions about exposure risk.

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Evaluating Mosquito Abundance Using a New Jersey Light Trap Fitted with an LED Light Bulb and BG Lure

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INTRODUCTION

For many decades New Jersey Light Traps (NJLT) that are equipped with an incandescent light bulb have been used for mosquito abundance monitoring programs. However, recently LED lights are being used in many parts of our everyday life, including mosquito traps. Encephalitis virus survey (EVS) traps have been fitted LED lights as secondary mosquito attractants for several years, likely because they draw little power. NJLTs also may benefit from being fitted with a 2.8-watt LED, because it produces 12% more light than the 25-watt incandescent bulb typically used in NJLT and uses up to 89% less electricity. Additionally, LED light bulbs have greater physical shock and shatter resistance. The BG-Lure increases the number of *Aedes aegypti* that are captured in BG sentinel traps and have the potential to increase the number of mosquitoes or diversity of species caught in NJLT. In this two-part study we determined whether substituting an incandescent light with an LED light bulb or adding BG-Lure affected the number of mosquitoes caught in EVS traps that were placed in a coastal habitat of Alameda County (California, USA).

METHODS

NJLTs fitted with an incandescent bulb, LED, or LED with BG-Lure were evaluated in three habitats: 1) tidal salt- and freshwater marsh, 2) suburban residential that was adjacent to a creek that flows throughout the year, and 3) wooded suburban residential. At each site, a pair of NJLTs were deployed 25 m from each other, with one fitted with the control (NJLT with 25 watt incandescent bulb) and the other with the treatment (NJLT with LED). A BG-Lure was affixed to the NJLT below the LED and directly above the fan by sliding the BG-Lure into a tube that was constructed of industrial strength plastic mesh (12.5 mm mesh size; see Poster). The NJLT ran continuously with the trap contents collected weekly. The location of the treatment and control was exchanged weekly at each site. The mosquitoes collected were identified to species and enumerated using a dissection microscope. The comparison of NJLT with an incandescent bulb or LED was made from 25 Apr to 12 Sep 2018 (84 trap nights), while the comparison of NJLT fitted with LED with or without BG-Lure was made from 28 Mar to 10 Oct 2018 (105 trap nights). Data from all study sites were combined and analyzed using Prism software (version 8; GraphPad Software, San Diego CA USA).

RESULTS AND CONCLUSION

NJLT fitted with an incandescent bulb caught slightly more female mosquitoes than one fitted with an LED bulb, but the difference was not significant (unpaired t test, $P = 0.73$; see Poster). Similarly, there was no significant difference in the number of male mosquitoes captured in NJLT fitted with an incandescent or LED light (unpaired t test, $P = 0.95$; see Poster). When NJLTs were outfitted with LEDs, more female mosquitoes were collected with the trap that contained a BG-Lure than with the trap without the BG-Lure, although this difference was not significant (unpaired t test, $P = 0.71$; see Poster). Similarly, there was no significant difference in the number of male mosquitoes collected in LED-fitted NJLT with or without BG-Lure (unpaired t test, $P = 0.82$; see Poster).

The results demonstrated that use of LED in NJLT is a good alternative to an incandescent light attractant. LEDs offered substantial advantages over incandescent light, most notably that the former draws far less power than LED lights, making their use more environmentally friendly relative to incandescent bulbs. Additionally, the life span of a LED far exceeds that of an incandescent bulb, providing additional cost savings. Vector control workers that replace incandescent lights with LED may be able to go a step further and retrofit the NJLT so that it is powered with sunlight (i.e. solar power), thereby eliminating the need for corded power. Doing so would substantially increase the diversity of sites for placing a NJLT (i.e. in the middle of a marsh where the lack of corded power would otherwise prohibit such placement). That there was no significant difference in the number of male and female mosquitoes collected in NJLT outfitted with incandescent or LED demonstrated that use of LED would still allow vector control workers to detect the initial emergence of male mosquitoes that typically precedes female emergence The addition of a BG-Lure did not impact on the number of mosquitoes collected by the NJLTs, demonstrating that in areas where invasive *Aedes* species have yet to be established (as was the case for Alameda County when the study was conducted), the additional expense of including a BG-Lure attractant on the NJLT is not warranted.

Exclusive Residential Exclusion

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Abstract

Exclusion is the single best Integrated Pest Management tool to minimize or eliminate unwanted animals at a residence such as raccoons, skunks, opossum, rats, mice, etc. Modification of the residential environment is essential to prevented unwanted animals that may decide to reside on your property. Items such as trees, shrubs, wood and compost piles may provide harborage for a variety of species. Our poster focuses on several basic exclusion systems which require foresight and innovation to achieve desired results.

Field Evaluations of Mosquito Gravid Trap Bait Solutions

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Introduction

The Reiter-Cummings modified gravid trap frequently is used by California mosquito and vector control agencies to collect adult gravid female mosquitoes for population monitoring and virus surveillance. A variety of attractant solutions are employed by agencies, but it is unknown whether these solutions vary in their ability to attract mosquitoes. The San Mateo County Mosquito and Vector Control District (District) laboratory evaluated six attractant solutions in the field to determine their relative effectiveness.

Methods

Site selection was based on the relative abundance of *Culex pipiens* at known trapping sites along the bayside of the San Mateo County peninsula. At the selected study sites, two different gravid trap infusions were deployed overnight per week at three-week intervals. The gravid traps were spaced ten feet apart along with a carbon dioxide-baited trap. Over the three week period, mosquito specimens collected were sorted by species and sex. In addition, all female mosquitoes were recorded as gravid, unfed, or blood-fed condition.

Results and Discussion

All gravid traps, regardless of solution recipe, attracted more gravid mosquitoes than CO_2 traps. Limited statistical tests were run on gravid trap collections from weeks 17-41 from four study locations: Joinville, Shorebird and Parkside north and south sites. Trap counts of gravid *Cx. pipiens* were significantly correlated with both the week trapped and the minimum overnight temperature (Pearson Product Moment Correlation, p-values <0.05). No significant differences were found between attractant solutions.

Conclusions

All evaluated bait solutions were effective at attracting gravid *Cx. pipiens*, with no single solution greatly outperforming the others. A district starting a new gravid trapping program might therefore prioritize other factors in choosing a bait solution such as cost, time, or targeted species. Our results encourage the future evaluation of other variables which may influence collection numbers, including site selection, trap design, and variations on bait fermentation time.

Acknowledgements

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Frequency and Distribution of Turkestan Cockroach Reports in Alameda County

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Abstract

Turkestan cockroaches, *Blatta lateralis* (Walker), are a relatively new invasive cockroach species in Alameda County. They are primarily an outdoor species that is usually found in water meter boxes, concrete pavement cracks, and near compost and vegetation. Turkestan cockroaches were first reported in the United States in 1978 in an army depot near Lathrop, California, in San Joaquin County and are thought to have been introduced via military equipment. Newer infestations in California are thought to be associated with accidental releases from Turkestan breeding colonies purchased from the Internet for use as pet reptile food. Despite Alameda County's proximity to San Joaquin County, the first confirmed introduction of *B. lateralis* was reported to the Alameda County Vector Control services District in 2013. In the 6 years since, there has been an increase in the frequency and distribution of service calls regarding Turkestan cockroaches. Herein, we mapped the frequency and distribution of Request For Service calls related to Turkestan cockroaches in Alameda County from 2013 to 2019 in relation to time of year, climate, and location.

Genomic epidemiology of West Nile virus in San Diego County

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Abstract

To investigate the drivers and dynamics of seasonal West Nile virus outbreaks in San Diego county, we sequenced 200 viral genomes from San Diego county as part of the WestNile4K (https://westnile4k.org/) project. Using Bayesian phylodynamic analyses, we were able to elucidate the patterns of transmission of the virus within San Diego County. We show that the central San Diego city region acts as a source and seeds viral lineages into other parts of the county. Using a combination of genomic and epidemiological data, we were able to quantitatively assess the contribution of environmental and ecological factors to West Nile virus transmission in San Diego. The high spatio-temporal sampling of viral genomes allowed us to show that the virus in able to successfully over winter within the county across multiple years. Our study shows how genomic epidemiology can be used on a county-level to investigate mosquito borne viral outbreaks and develop effective mitigation strategies.

Geospatial Response Identification System (GRIdS): Developing a Risk-Based Driven West Nile Virus Response Grid System using ArcGIS Hot-Spot Analysis

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Abstract

Following Orange County's worst West Nile virus (WNV) epidemic in 2014, Orange County Mosquito and Vector Control District (OCMVCD) has taken steps to increase early detection of focused WNV virus activity and decrease control response time. In 2019, OCMVCD implemented a Geospatial Response Identification System (GRIdS) derived from 15 years (2004 – 2018) of location data for WNV human cases and virus positive dead birds. OCMVCD used this information to construct a hot-spot analysis to spatially identify three County regions based on WNV risk: high, medium, and low, with a goal of distributing surveillance resources more efficiently. Each area was divided into blocks (one gravid trap per block) with dimensions set to accommodate OCMVCD's ground based adulticiding resources. Each block was further divided into smaller cells to delineate sections for additional weekly mosquito sampling that was activated upon an initial WNV detection. The area with the highest density of WNV activity, based on human cases and dead birds, was assigned a tighter surveillance grid compared to the medium and low risk areas. Within each risk area, the blocks established a standard unit for calculating infection rates based on routine and extended trapping efforts. During 2019, the grid system proved to enhance the communication of risk to constituents and stakeholders at a neighborhood level. The grid system and surveillance plan further strengthened inter- and intra-agency communication allowing for a more rapid control response.

New Cost-Effective “Tip Cards” For Disseminating Vector Control Information

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Abstract

Brochures are one tool that the San Diego Vector Control Outreach Program uses to distribute important educational and contact information to the public. However, we noticed that our main 8.5 × 4 inch trifold Invasive *Aedes* brochures and 8.5 × 5.5 inch half page West Nile virus brochures were not being picked up frequently by visitors to our booth at events. Online materials were preferred by many customers; however, a quick reference paper-based informational material was still needed, especially for those with limited online access. To address this, we redesigned our brochures into a series of smaller, colorful, and graphic 6 × 3.5 inch “tip” cards that are easier to distribute and provide useful information more through pictures than words. These new cards are cheaper to produce and are picked up more frequently by attendees at our events than our older, larger and text-heavy brochures. We present the process behind the new designs, their cost efficiency, and how they compare to the older brochures.

Novel social media approaches to gain influence and awareness in the District

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Abstract

Social media is an inexpensive, effective resource for communicating with residents. In the last year, the West Valley MVCD (District) has put forth a concerted effort to use social media to promote prevention and inform our residents of current disease risk. The District has created several campaigns to increase social awareness of vector control, disease activity, mosquito populations, and resident responsibility. Through infographics, weekly updates, and interactive events, the District has grown its social media presence greatly in the past 2 years. The District plans to continue to use the power of social media to keep residents up to date and engaged with vector control.

Observing efficacy of Sumilarv 0.5G, FourStar Bti CRG, VectoMax WSP, Natular G30 WSP, and Natular XRT treatments in catch basins

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Introduction

Using extended release larvicides to control mosquito larvae is a time saving choice, but when a product does not persist for the expected length of time, the result is the emergence of adult mosquitoes which causes an increased risk of virus transmission. Because it is operationally important to determine the length of effectiveness of products in real-world applications, we evaluated five products in current or planned use by our District in storm drain catch basins (CB). A similar study was conducted in 2018 by our District (Erspamer et al. 2019) wherein four extended release briquette formulations were evaluated. The current study evaluated a variety of products designed for use in CB including: two water soluble pouches, one controlled release granules, one tablet, and one sand-based insect growth regulator.

Methods

The 115 CB selected for this study were spread throughout two cities in our District, Alameda and Berkeley, [see Poster] and were chosen because they were historically known to contain stagnant water and produce mosquitoes. We did not include untreated control CB in for this study. For the fifteen CB treated with Sumilarv 0.5G (MGK, Minneapolis, MN), pupal samples were collected pretreatment to evaluate normal adult emergence before applying 75 grams of product. Post-treatment pupal samples were collected weekly and brought to the laboratory in glass jars to measure adult emergence. The other 100 CB were divided as follows: 25 CB each were treated with 20 grams of FourStar Bti CRG (FourStar Microbial Products LLC, Schaumburg, IL) each, 25 CB each were treated with 1 VectoMax WSP (Valent BioSciences, Libertyville, IL), 25 CB each were treated with 1 Natular WSP (Clarke, St. Charles, IL) and 25 CB each were treated with 1 Natular XRT (Clarke, St. Charles, IL). After treatment, these CB were inspected every two weeks for the presence of larvae. Once larval instars later than 2nd (Harbison et al. 2017) were found, the treatment was considered to have failed and the CB was treated with an alternate product and removed from the study. If a CB dried out, it also was removed from the study and statistics.

Results and Discussion

The four larvicide products that were tested showed shorter effectiveness than indicated by the product label (Graph 1; see Poster). FourStar Bti CRG has a labeled efficacy duration of 40 days; however, 2 wks post-treatment 4 [16%] of 25 CB contained larvae; 4 wks post-treatment an additional 1 [4.8%] of 21 CB contained larvae [see Poster]. VectoMax WSP has a labeled efficacy length of 42-56 days; however, 2 wks post-treatment 2 [8.3%] of 24 CB contained larvae; at 4 wks post-treatment an additional 5 [22.7%] of 22 CB contained larvae, and at 6 wks post-treatment 8 [66.7%] of the remaining 12 CB contained larvae. Natular WSP has a labeled efficacy length of 30 days; however, 2 wks post-treatment 1 (4.3%) of 23 CB contained; at 4 wks post-treatment 5 [26.3%] of the remaining 19 CB contained larvae. Natular XRT has a labeled efficacy length of 180 days; however, 2 wks post-treatment 2 (8.3%) of 24 CB contained larvae, 4 weeks post-treatment resulted in 4 out of 21, or 19%, CB containing larvae, 6 weeks post-treatment resulted in 9 out of 13, or 69.2%, CB containing larvae (see Poster). Remaining CB were dry before the study was complete. There was no significant difference in the proportion of CB that contained larvae for CB treated with Natular XRT or Vectomax WSP (unpaired t test, $P = 0.9742$) and Natural G30 WSP or FourStar Bti CRG (unpaired t test, $P = 0.9550$). The granule formulations of FourStar and Natular seem to eliminate larvae more effectively compared to their large briquet versions (Erspamer et al. 2019) (Harbison et al. 2017). Sumilarv 0.5G has a labeled efficacy length of 28-35 days. Sixteen weeks of sampling field CB resulted in only 25 adults (0.79%) emerged from 3,156 pupae evaluated (Graph 2; see Poster). Linear regression of the Sumilarv 0.5G data, with 0 days post treatment values removed, showed that the slope was not significantly different from 0 ($F = 0.806$; $= 1, 15$; $P = 0.37$), indicating no loss in efficacy over the 16 wks of evaluation.

Conclusion

Field technicians heavily rely on product labels when deciding which material to use and when to retreat. If a treatment does not control mosquito larvae for as long as expected, the technician may not realize this failure, allowing the emergence of adult mosquitoes. The current study emphasized the importance of regular post-treatment inspections and the choice of long-lasting effective products for each treatment.

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Preliminary Data on the Performance of BG-Sentinel and CDC-AGO Traps for *Aedes aegypti* in a Stockton Suburb

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Abstract

Aedes aegypti is an invasive species of mosquito that is a competent vector of several important pathogens such as dengue, Zika, and yellow fever viruses. It was first detected in San Joaquin County on August 8, 2019 in the Boulder Creek suburb in Stockton, CA. Several trap types were used to estimate *Ae. aegypti* abundance, including the BG-Sentinel and CDC-Autocidal Gravid Ovitrap (CDC-AGO). The BG-Sentinel uses a combination of visual cues, convection currents and chemical odors, and primarily attracts host-seeking females, whereas the CDC-AGO is normally baited with hay-infused water to attract gravid females. A variety of underlying factors, including mortality during the gonotrophic cycle, could contribute to discrepancies in collected adults via host-seeking traps and gravid traps. The purpose of this study was to compare these two traps by placing them in close proximity at four locations, one of which being the index house where *Ae. aegypti* was discovered. The BG-Sentinels were baited with BG-lures containing human chemical cues, in addition to CO2 dry ice. The CDC-AGOs were baited with hay-infused tap-water (approximately two gallons). At the index house where abundance was high, the BG-Sentinel collected more adults than the CDC-AGO. When adult numbers were less than 10, either trap could collect adults in similar quantities. When adult numbers were less than two, the BG-Sentinel was more likely to collect adults than the CDC-AGO. Overall the data indicated that BG-Sentinels were more effective for estimating *Ae. aegypti* abundance in San Joaquin County. CDC-AGOs may be used to supplement BG-Sentinels, and can detect the presence or absence of *Ae. aegypti* in a given area of surveillance.

Pyrethroid resistance in *Culex quinquefasciatus* (Diptera: Culicidae) from different ecological niches in West Valley Mosquito and Vector Control District

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Abstract

The application frequency and amount of pyrethrins and pyrethroids (the mimics of natural pyrethrins) applied depend on the distinctive purposes and pest targets among urban, suburban, agricultural, and riparian ecozones in the West Valley area of San Bernardino County, California. Urban is referred to an area with dense human dwellings. Suburban is described as a place where most of the industrial activities and factories are concentrated. Agriculture refers to the farms and dairies, and riparian is the interface between land and a river or stream near the Prado Basin. The purpose of this project was to compare the susceptibility of the southern house mosquito, *Culex quinquefasciatus* Say, from the field to the laboratory reference colony of the same species. The times to 50 (LT_{50}) and 90% (LT_{90}) motality to technical permethrin in all populations were determined by standard bottle bioassay. It was noticed that field populations from various ecozones showed distinct levels of tolerance or resistance. The resistance ratios (RR) based on LT_{50} ranked suburban (3.72-fold) > urban (3.16-fold) > agricultural (2.52-fold) > riparian (1.86-fold). The RRs based on LT_{90} ranged urban (15.10-fold) > suburban (9.84-fold) > agricultural (6.25-fold) > riparian (3.77-fold). The resistance levels and their differences among various ecozones were discussed according to presumed application patterns and residue distribution of pyrethroids. The findings are useful to direct adulticiding for mosquito control and resistance management using pyrethroids.

The Apex blood-feeding system: a simple, lower-cost apparatus for feeding hematophagous insects

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Introduction

Maintenance of anautogenous mosquitoes and other hematophagous insects requires a source of warm mammalian or avian blood. Although living animals or human volunteers can be used, there are potential ethical, legal and logistical issues which can make this problematic. Apex Bait Technologies has developed a simple, cost-effective blood feeding system which we have used successfully to maintain a *Culex tarsalis* colony.

Methods

The Apex feeding system consists of a temperature control unit and a heating coil and thermocouple embedded in a small aluminum block with a machined reservoir for holding approximately 5 ml of blood. The temperature control unit maintains the block and the blood at a specific temperature (we set ours at 37°C). A piece of Parafilm® stretched over the reservoir serves as an 'artificial membrane' which the insects can pierce to obtain a blood meal. We fed our colony with whole chicken blood with sodium EDTA; blood was frozen in aliquots and thawed just prior to use.

Results and discussion

We successfully used the Apex system to establish and maintain a colony of *Culex tarsalis* (Bakersfield strain, obtained from Sacramento-Yolo MVCD) during 2019. Females are rapidly attracted to the unit once it is placed in their cage, and engorge quickly. The percentage of engorged females and subsequent egg raft production was far superior to our previous experience with the same mosquito strain and an animal (quail or mouse) or human volunteer blood source. For best results, we found that sugar sources should be removed 24 hours prior to blood feeding

Conclusion

Although there are other commercial feeding systems available, we found the Apex system to be simple, reliable, cost-effective and easy to use and it has become our method of choice for maintaining anautogenous mosquito colonies.

Acknowledgement

Thanks to Deborah Dritz and the laboratory staff at the Sacramento-Yolo Mosquito & Vector Control District for providing egg rafts for the re-establishment of our *Culex tarsalis* colony.

Identification of cryptic mosquito eggs using conventional PCR with extraction protocol recommendations

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Abstract

Prompt identification of container-breeding *Aedes* mosquito species (*Ae. aegypti*, *Ae. albopictus* and *Ae. notoscriptus*) is vital in mosquito control programs to prevent the establishment of these invasive species in non-native habitats in the United States. We have adapted a conventional Polymerase Chain Reaction (PCR) assay for use in invasive *Aedes* mosquito surveillance programs that will allow for relatively rapid identification of 'cryptic' mosquito specimens or mosquito eggs. This assay provides an alternative to rearing eggs, which can be difficult in laboratory settings, or relying on identification of damaged specimens with missing diagnostic features.

Introduction

Invasive *Aedes* species (notably *Ae. aegypti*, *Ae. albopictus* and *Ae. notoscriptus*) have been spreading rapidly throughout the state of California, since a thriving population was detected in Los Angeles county in 2011 (Metzger et al. 2017). Over the past nine years, early detection of these species has become a principle component of many mosquito control and surveillance programs striving to eliminate these emergent populations (Mehta et al. 2007). Current methods for detecting the presence of invasive *Aedes* mosquitoes require either the successful capture and identification of adults, or the successful rearing of wild-collected larvae or eggs. Although these methods are considered definitive for detecting invasive *Aedes*, there are many drawbacks which may delay or prevent early detection. First, mosquito eggs collected from the field must be kept viable and held under specific conditions in order to hatch. Second, successfully hatched first instar larvae must survive until diagnostic characters develop in older larvae. Third, staff must be sufficiently trained to be able to correctly identify invasive *Aedes* spp. among native species with similar ecological preferences (e.g. *Ae. sierrensis*). Even with perfect execution, the successful rearing of mosquito eggs may take days or weeks, which may delay treatment and surveillance efforts that could have prevented further spread and establishment.

The Cytochrome Oxidase 1 gene (CO1) has been used widely in molecular genetics as a barcoding gene, because it mutates at a predictable rate that allows a reasonable differentiation of species (Simon et al. 1994, Kress and Erickson 2008). Large databases of genetic sequences exist for invasive *Aedes* for this gene region (Soni et al. 2018, Afizah et al. 2019) and the primers and assays already have been well-developed, making it ideal for use. The adaption of a CO1 PCR assay for in-house use for mosquito control districts will provide a surveillance tool to rapidly identify suspected invasive *Aedes* eggs, or unidentifiable adult specimens within as few as two days.

Materials and Methods

Development of CO1 Assay Using Adult Mosquito Samples

Whole adult *Aedes sierrensis* (n=8) and *Culex pipiens* (n=7) mosquitoes were collected from the field during routine surveillance. All individuals were identified in the laboratory using the California specific dichotomous key (Meyer and Durso 1998), then pooled by species in reinforced 2-mL tubes with five ceramic beads and 700 μL of Lysis/Binding Solution Concentrate (Applied Biosystems, Waltham, MA) diluted with an equal volume of 100% isopropanol added to each. Tubes were physically disrupted for 30 sec using an Omni Bead Ruptor (OMNI International, Kennesaw, GA) tissue homogenizer. Nucleic acid purification was conducted using the MagMax Viral RNA Isolation Kit (Applied Biosystems) according to the manufacturer's recommendations for semen.

Primers for the CO1 assay were obtained from Simon et.al. 1994. The forward primer was: *CO1F* 5′ – GGAGGATTTGGAAATTGATTAGT-TCC - 3′; and the reverse primer was *CO1R*: 5′ – ACTGTAAATATAT-GATGTGCTCA – 3′. The 25 μL PCR reaction contained 3 μL of DNA template, 5 μL of Phusion High-Fidelity 5x Buffer (New England Biolabs, Ipswitch, MA), 0.5 μL dNTP's (ThermoFisher, Waltham, MA), 1.25 μL of each primer (10 μM), and 0.25 μL of Phusion High-Fidelity DNA Polymerase. Cycling conditions were obtained from Afizah et al. (2019) with the following modifications:

Table 1.—Commercially Available DNA Extraction Methods Compared

Extraction Kit	Vender	Disruption Method	Tune Incubated	Incubation Temoerature	Lvsis A11:ent	Lvsis Buffer
Qiagen DNeasy Blood and Tissue	Qiagen	Pestle	24 hours	56°C	Proteinase K 20 mgimL	ATL
MagMax Viral RNA Isolation Kit	Thermofisher Scientific	OMNI	0 hours	NA	Lysis Binding Solution	Lysis Binding Solution
MagMax Viral RNA Isolation Kit	Thermofisher Scientific	OMNI	120 hours	4°C	Lysis Binding Solution	Lysis Binding Solution
Qiagen DNeasy Blood and Tissue	Qiagen	OMNI	2 hours	56°C	Proteinase K 100mgimL	PK buffer
NucleoSpin DNA Insect Kit	Takara	OMNI	0 hours	NA	Proteinase K	BE buffer
NucleoSpin DNA Insect Kit	Takara	Vortex	20 min	Ambient	Proteinase K	BE Buffer

initial denaturation was 98°C for 30 sec, followed by 30 cycles of 98°C for 10 sec, 45°C for 15 sec and 72°C for 15 sec. The final extension was 72°C for 10 min. Samples were amplified in-house using a T100 thermocycler (Bio-Rad, Hercules, CA) and the resultant product was run on an electrophoretic gel with the expectant size approximately 610 bp. Positive runs were purified using Agencourt AMpure XP for PCR Purification assay (Beckman Coulter Life Sciences, Indianapolis, IN) and submitted to ELIM Biopharmaceuticals Incorporated in Hayward, CA for sequencing in both the forward and reverse directions. Sequences were aligned using UGene: Integrated Bioinformatic Tools (Unipro, Russia) and compared to sequences obtained from the National Center for Biotechnology Information Database (NCBI) for species identification.

Optimization of Mosquito Egg Extraction

Mosquito eggs were acquired from the San Mateo County Mosquito and Vector Control District laboratory colony of *Culex pipiens* (71^{st} generation). Freshly laid eggs (24 h or less) were removed from cups of filtered water, vortexed in 70% ethanol for 10 sec to remove surface contaminants, and placed in 1.5 mL sterile tubes or 2.0 mL reinforced tubes depending on the extraction protocol. We ran a brief test using no physical disruption technique; however, after 24 h of incubation at 56°C with ATL buffer and Proteinase K (20 mg/mL) there was no apparent reduction in egg integrity. Further experimentation used either a pestle in a 1.5 mL tube, running the eggs for 30 sec in the OMNI tissue homogenizer, or placing 2.0 mL tubes with sample and lysis solution in a vortex mixer fitted with a 24-microtube holder and shaking them on the highest setting for 20 min.

Chemical/enzymatic nucleic acid purification kits under examination included: Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), MagMax Viral RNA Isolation Kit, the NucleoSpin DNA Insect Extraction Kit (TaKaRa Bio Inc.), and Qiagen DNeasy Blood and Tissue Kit substituting the ATL buffer and the Proteinase K (20 mg/mL) provided in the kit with 92 µL PK Buffer and 8 µL of 100 mg/mL Proteinase K (ThermoFisher Scientific) per sample as recommended by Nieman et.al. (2015) for maximum DNA yield (Table 1). The instructions provided with the kits were followed exactly as written unless otherwise noted. Three replicates were conducted for each nucleic acid purification kit using one, three, or five eggs (except for the NucleoSpin Kit where extractions using 5 eggs were not run). After purification, DNA yield was analyzed using a Qubit fluorometer (ThermoFisher Scientific) and all templates were amplified using the optimized CO1 assay, sent to ELIM Biopharmaceuticals for sequencing, and analyzed as described above.

Results

Physical disruption results were consistently more homogeneous using the OMNI tissue homogenizer compared to grinding with a pestle. This led to shorter incubation periods required for enzymatic digestion for the Qiagen DNeasy Blood and Tissue Kit (2-hours vs. 24-hours). The NucleoSpin DNA Insect Kit and the MagMax Viral Isolation Kit did not require an incubation period and so could not be compared here. The Qiagen DNeasy Blood and Tissue Kit and the NucleoSpin DNA Insect Kit proved to consistently have higher DNA yield than the MagMax Viral Isolation Kit (Table 2). This result could not statistically evaluated because DNA yield from the MagMax Kit was often too low to be calculated using the Qubit fluorometer. The use of the Thermofisher PK buffer and Proteinase K (100 mg/mL) in place of the Qiagen ATL buffer and Proteinase K (20 mg/mL) produced consistent DNA yield although the yield was not significantly greater than the with other methods examined ($p=0.82$).

Conclusions

We optimized a Cytochrome Oxidase 1 conventional PCR assay to rapidly identify cryptic mosquito specimens or mosquito eggs that may belong to invasive *Aedes* species. This assay provides an alternative to rearing eggs, or relying on accurate identification of damaged specimens with missing diagnostic features. Although conventional PCR allows for the identification of insect specimens, it should not be used exclusively as a means of identifying invasive species. Early surveillance trapping

Table 2.—DNA yield results and amplification success rates for mosquito eggs using various extraction techniques

Extraction Technique	Disruption Method	Number of eggs	Qubit Highest Result (ng/µL)	Qubit Lowest Result (ng/µL)	Number of replicates successfully amplified
Qiagen Blood and Tissue	Pestle	1	0.04	0.032	3/3
		3	0.916	0.0432	3/3
		5	0.056	0.0232	3/3
MagMax Viral RNA, 700 µL Lysis Binding Solution	OMNI	1	Too Low	Too Low	3/3
		3	Too Low	Too Low	3/3
		5	0.0224	Too Low	3/3
MagMax Viral RNA 450 µL Lysis Binding Solution	OMNI	1	Too Low	Too Low	1/3
		3	Too Low	Too Low	3/3
		5	Too Low	Too Low	1/3
Qiagen with Life Sciences PK buffer and Proteinase K {00mg/mL)	OMNI	1	0.151	0.0264	3/3
		3	0.436	0.156	3/3
		5	0.656	0.348	3/3
NucleoSpin DNA Insect Kit	OMNI	1	0.024	0.0196	3/3
		3	0.0792	0.0676	3/3
NucleoSpin DNA Insect Kit	Vortex	1	0.0188	0.0104	3/3
		3	0.042	0.0374	3/3

and collection methods, including, but not limited to, the use of ovi-traps, BG-Sentinel traps (Biogents, AG), CO_2-baited EVS-style traps, and property inspections, should always be deployed in conjunction with laboratory confirmation to assess the scope of the invasion and the degree to which the invasive species has been established. A qPCR multiplex assay would be highly desirable in order to further reduce the length of time necessary to identify invasive *Aedes* eggs. Such an assay would be more accessible to vector control agencies who may not be able to afford new equipment, materials, or sequencing services, or who do not retain staff that are comfortable with conventional PCR.

Acknowledgments

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The West Nile 4K Project: Reconstruction of the Spread and Evolution of West Nile virus in the United States

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Abstract

Since its emergence nearly 20 years ago, West Nile virus (WNV) has become the most important mosquito-borne virus in the United States. Cycles of West Nile virus outbreaks have led to an estimated 7 million human infections and have decimated certain bird populations. Even though there are expansive WNV surveillance and vector control systems in place, our ability to reduce the burden of disease is hindered by our lack of a detailed understanding of what drives outbreaks. For instance, it is unknown how WNV spreads regionally and nationally, what facilitates it's spread, if it is adapting to local mosquito populations, and how genetic diversity contributes to outbreaks. To answer these questions, we formed the West Nile 4K Project which will incorporate genomic approaches into traditional epidemiological and experimental investigations to yield deep insights into how WNV emerges, spreads, and evolves. The project is generating a large genomic dataset by sequencing thousands of WNV genomes from samples provided by our large network of collaborating public health partners across the United States, and will directly examine the impact of WNV genetics on virus fitness and adaptation using a series of controlled laboratory experiments. We will obtain a high definition reconstruction of WNV spread and evolution, allowing us to define regional WNV transmission networks, determine the role of virus evolution in local outbreaks, and examine the impact of WNV genetics on virus fitness and adaptation. Additionally, by sharing our data and analyses in real-time, we are providing up-to-date insights with our public health partners and the academic community to inform WNV control measures.

Use of a Pop-Up Garden Waste Bag as a Resting Mosquito Trap for Mosquito Surveillance

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INTRODUCTION

Mosquitoes in marsh habitats blood feed predominantly on wildlife where they may acquire and transmit arboviruses such as West Nile virus (Molaei et al. 2010). Carbon dioxide baited encephalitis virus survey (EVS) traps typically capture host-seeking mosquitoes, with blood-fed and gravid mosquitoes rarely present in this type of trap. As the blood-fed and gravid mosquitoes are more likely to be infected with arboviruses compared to those that are host-seeking, we sought to determine whether a resting box trap would improve our catch of virus-infected mosquitoes.

METHODS

Construction of the human-powered resting shelter trap that was described previously (Burkett-Cadena et al. 2019) was time consuming and laborious. Particularly vexing was lining the entire surface area of the trap with plastic and producing the mosquito capture lid from a plant container (Burkett-Cadena et al. 2019). As a result, we sought ready-made products that were similar to the resting shelter trap mentioned above and found that a 45-gallon pop up yard waste bag and a plastic garbage can lid available online or a local hardware store were efficient alternatives. The pop-up yard waste bag measuring 27 inches tall and 22 inches in diameter was made of durable canvas material, black on the interior with a green exterior. Thus, the shape and color were similar to what was described previously (Burkett-Cadena et al. 2019); however, it was somewhat larger. The mosquito collection apparatus (MCA) was manufactured by cutting a 4.5-inch diameter hole into the center of a 22-inch plastic lid and fitted with the funneled end of a mosquito breeder apparatus (model 1425; Bioquip, Rancho Dominquez CA). A large black wet bath towel (52x29 inches) was placed inside the RMT to produce a humid environment that encouraged mosquitoes to enter the trap and to increase the mass of the RMT so that it was less likely to be moved by wind or animals. RMT were deployed for at least 4 days prior the first collection date so that mosquitoes became acclimated to its presence in the area. To capture mosquitoes, the MCA was rapidly placed over the front opening, the RMT rotated with the MCA facing upward and the RMT pressed down rapidly at least 4 times to expel the mosquitoes from the RMT into the collection chamber of the MCA (Poster).

RESULTS AND CONCLUSION

Using the 45-gallon pop-up Garden Waste Bag as a RMT we were able to capture gravid and blood fed and male mosquitoes. A substantial advantage of the RMT over many other mosquito trap designs is that our design does not require electrical power. The RMT collected 14 of the 22 mosquito species that commonly occured in Alameda County during the spring of 2019 (Poster). Over 325 trap nights, the RMT captured a total of 1,131 (3.48 mosquitoes / trap night). We found a similar proportion of male and female mosquitoes collected in the RMT with no species bias. However, there was more *Cx. tarsalis* male mosquitoes in the traps as opposed to females . Most of the female mosquitoes that were captured in the RMT were either blood-fed or gravid .

The RMT is an inexpensive and simple to use trap. It is one quarter the cost of buying a commercially available resting box trap and it can be easily integrated into existing mosquito monitoring programs by simply placing it at EVS trap sites and collecting the contents of the RMT while picking up the EVS trap.

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Yeast fermentation as a cost affordable CO_2 source for BG-Sentinel traps

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INTRODUCTION

BG-Sentinel traps are an integral part of mosquito and arbovirus surveillance for host-seeking *Aedes* mosquitoes (AFPMB 2012). These traps used in conjunction with a BG-Lure® and a form of carbon dioxide (CO_2) are considered the gold standard for sampling *Aedes* and are widely-used by a variety of mosquito control and public health agencies worldwide to assess temporal and spatial distribution (Nguyen et al. 2010, Whelan et al. 2011). The forms of CO_2 commonly used with the BG-Sentinel trap include dry ice, compressed gas, and yeast fermentation (Smallegange et al. 2010). When conducting a large-scale mosquito surveillance program, the CO_2 source used is dependent on budget, weather, and accessibility to CO_2. Since 2018, Delta Vector Control District (DVCD) has deployed 84 BG-Sentinel traps a week using a BG-Lure® and yeast fermentation mixture as the CO_2 source. The current study compared cost and effectiveness of yeast fermentation to dry ice as a CO_2 source for BG-Sentinel traps.

METHODS

The yeast fermentation mixture was modified from Saitoh et al. (2004) and contained sugar (250 g), active dry yeast (17.5 g), and water (2 L). The contents were poured into a plastic container (3.78 L) with a 1.27 cm diameter vinyl tube (0.61 m) attached through the cap. Dry ice was added in a similar way to modified dry ice-baited, Centers for Disease Control and Prevention (CDC)-style encephalitis vector survey (EVS) traps (Rode and Fall 1979). A dry ice block (2.27 kg) was crushed into large pieces that fit into an insulated (R 3.5) paint can (3.78 L) with a 1.27 cm diameter vinyl tube (0.61 m) attached through the side of the can (Poster, Fig. 1).

BG-Sentinel 2 traps were set Monday to Thursday at three suburban locations with established *Aedes aegypti* (L.) populations from August 26 to October 3, 2019. The traps were checked daily to collect adult mosquitoes and rotate the CO_2 treatments (yeast fermentation, dry ice, or no CO_2) among locations. Traps also were rotated throughout the study, but set in the same spots at each location, and powered using an AC wall adapter. New BG-Lures® were unwrapped for the first trap set and used throughout the duration of the study. The data recorded included adult mosquitoes enumerated by species, location, and type of CO_2 source for each trap set. Statistical significance ($P<0.05$) was calculated using analysis of variance (ANOVA) and post-hoc Tukey Test in RStudio statistical software (R version 3.6.1).

Figure 1.—Dry ice container, BG-Sentinel 2 Trap, and yeast fermentation container.

RESULTS AND DISCUSSION

A total of 69 trap sets were recorded over five weeks, with 23 sets at each location. The first null hypothesis tested was that all three locations caught the same number of mosquitoes to ensure no location bias. Location 1 captured 193 mosquitoes with 105 being female *Ae. aegypti*, location 2 captured 242 mosquitoes with 149 being female *Ae. aegypti*, and location 3 captured 264 mosquitoes with 80 being female *Ae. aegypti*. Even though meteorological factors and changes in nearby mosquito sources may have influenced the results, a one-way blocked ANOVA indicated that the trap locations were not significantly different from one another ($F=0.889$; $DF = 2, 66$; $P=0.416$).

The second null hypothesis tested was that yeast fermentation, dry ice, and no CO_2 source caught the same number of mosquitoes and female *Ae. aegypti*. The total counts for the 23 trap sets in each configuration were as follows: dry ice captured 396 mosquitoes with 207 being female *Ae. aegypti*, yeast fermentation capturing 252 mosquitoes with 101 being female *Ae. aegypti*, and no CO_2 source capturing 51 mosquitoes with 26 being female *Ae. aegypti* (Poster, Fig. 2). ANOVA indicated that the CO_2 sources did not catch the same number of mosquitoes

(F=48.77; DF = 2, 66; P<0.001) or female *Ae. aegypti* (F=26.31; DF = 2, 66; P<0.001). A post-hoc Tukey test indicated that dry ice caught significantly more mosquitoes (P<0.001) and female *Ae. aegypti* (P<0.001) than yeast fermentation, and yeast fermentation caught significantly more mosquitoes (P<0.001) and female *Ae. aegypti* (P=0.011) than no CO_2 source, and dry ice caught significantly more mosquitoes (P<0.001) and female *Ae. aegypti* (P<0.001) than no CO_2 source.

Overall, results from this study align with past research and indicate that BG-Sentinel traps follow a similar trend to CDC light traps and EVS traps when using dry ice or yeast fermentation as the CO_2 source.

One season for DVCD requires the materials for 21 containers and setting 84 traps per week from April to October (26 weeks). To run this large-scale invasive *Aedes* surveillance program for one season using BG-Sentinel traps with yeast fermentation as the CO_2 source costs $920.01

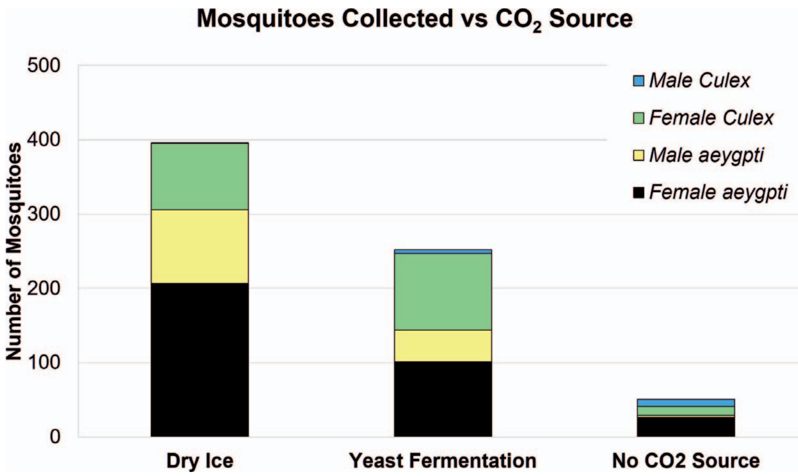

Figure 2.—Comparison of the total number of mosquitoes caught from August 26th to October 3rd for dry ice (female *Ae. aegypti* 207, male *Ae. aegypti* 99, female *Culex* 89, male *Culex* 1), yeast fermentation (female *Ae. aegypti* 101, male *Ae. aegypti* 43, female *Culex* 103, male *Culex* 5), and no CO_2 source (female *Ae. aegypti* 26, male *Ae. aegypti* 3, female *Culex* 12, male *Culex* 10).

These results agreed with related studies (Saitoh et al. 2004, Oli et al. 2005, Smallegange et al. 2010, Aldridge et al. 2016) that showed dry ice captured significantly more mosquitoes than yeast fermentation, and yeast fermentation captured significantly more mosquitoes than no CO_2 source. Saitoh et al. (2004) found that sublimating dry ice produced 12 times more CO_2 than their yeast fermentation mixture. This finding is in agreement with Reisen et al. (2000) that the amount of CO_2 released has an important role in determining how many mosquitoes are captured.

with each yeast fermentation mixture costing $0.36 and one container $6.37. In contrast, to run the same program using dry ice as the CO_2 source costs $14,488.95 with dry ice for one trap costing $6.57 and each container $6.67 (Poster, Fig. 3). Thus, one season of using yeast fermentation instead of dry ice as the CO_2 source saves $13,568.94 a year. For mosquito control and public health agencies with a small budget, this becomes a factor to consider if the cost savings of yeast fermentation outweighs the trapping effectiveness of dry ice as a CO_2 source for BG-Sentinel traps.

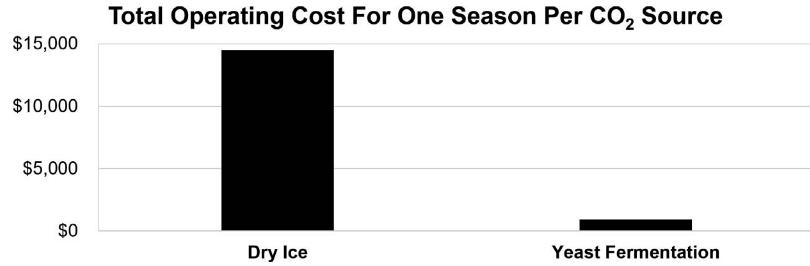

Figure 3.—Comparison of total operating cost for one season, including materials and contents for 84 traps sets per week for 26 weeks, for dry ice ($14,488.95) and yeast fermentation ($920.01).

CONCLUSIONS

The DVCD has now completed the second season of using yeast fermentation as the CO_2 source with BG-Sentinel traps as part of the District's invasive *Aedes* surveillance program. Though proven to be not as effective as dry ice, yeast fermentation has proven to produce enough CO_2 to attract *Ae. aegypti* in suburban areas. Due to budget constraints and low supply of dry ice within our district, DVCD plans to continue using yeast fermentation as the CO_2 source with BG-Sentinel traps. The cost savings of $13,568.94 a year allows the District to set more traps than it could with dry ice. This increase in trap spatial surveillance improves monitoring of *Ae. aegypti* populations across the District. Therefore, field mosquito technicians can be used accordingly to prioritize control efforts at new and existing locations. Further studies are being conducted to test temperature, mixture ratios, and lifespan of the yeast to optimize the amount of CO_2 produced by the yeast fermentation process.

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Comparison of trap collections and cost of commercially available and homemade yellowjacket traps in Lake County, California

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Abstract

Yellowjackets are notable pests of humans due to their opportunistic foraging behaviors, painful stings, and potential for causing dangerous allergic reactions. Baited traps provide a useful supplement for controlling yellowjackets compared with nest treatments, which are often dangerous, time consuming, costly, and do little to prevent nuisance interactions between humans and foragers. Our study compared three homemade yellowjacket traps and three commercially available traps in Lake County, California to determine efficacy and cost-benefit. Traps were set at five sites and randomly rotated among six plots per site, and baits were changed every two weeks per commercial manufacturer recommendations. Cost benefit was determined using material and bait cost as well as bait change frequency and overall trap efficacy. Yellowjacket count data was analyzed using a hurdle model. Traps compared included the Rescue!® Yellowjacket trap, the Rescue!® Wasp, Hornet, and Yellowjacket trap, the Victor® Yellowjacket trap, a homemade bottle trap, a jar trap, and a homemade jug trap. The total number of yellowjackets collected was 33,321. The trap that collected the highest number of yellowjackets was the Rescue!® Yellowjacket trap (n=19,257) and the trap that collected the fewest yellowjackets was the jar trap (n=65). The Rescue!® Yellowjacket trap was the most cost-effective, calculated at approximately $0.40 per 100 yellowjackets collected. The jar trap was the least cost-effective, calculated at approximately $31.10 per 100 yellowjackets collected. The Rescue!® Yellowjacket trap was overall the most effective and cost-efficient trap evaluated in Lake County.

Prioritizing door-to-door yard inspections based on prior breeding status to control *Aedes aegypti*

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INTRODUCTION

Although source reduction is considered the most effective means to reduce mosquito populations, *Aedes aegypti* (L.) preference for small, man-made containers and other cryptic sources make these efforts difficult to sustain (AMCA 2017, CDC 2017, CDPH 2019). Successful source reduction requires the strategic use of limited resources and active community participation (Morrison et al. 2008, Achee et al. 2015).

Since 2017, Delta Vector Control District has conducted property inspections for *Ae. aegypti* sources by homeowner request or when the number of females per trap-night reached a predetermined threshold for that year. During an inspection, field mosquito technicians search for sources, eliminate or treat any they find, and educate residents on control methods for existing or potential sources on their properties. Through this process, residents ideally learn to practice control methods and then apply this knowledge in subsequent mosquito seasons to prevent mosquito production on their property. Although residents often controlled sources during the year, it was unclear if they continued to implement control practices across multiple years. It also was unclear if the inspection process could help prevent residents from developing mosquito sources in the future, if active sources were not found on their property. The current evaluation was conducted to clarify if residential inspections, regardless of actual findings, have a lasting impact across multiple years and to determine if future property inspections can be prioritized based on the presence or absence of *Ae. aegypti* in prior years.

METHODS

From 2017 to 2019, 2,420 unique properties were inspected for *Ae. aegypti* sources. The data were categorized each year by assigning a property status of "Breeding" if mosquito larvae were found, "Not Breeding" if no larvae were found, or "N/A" if no inspection was conducted. Properties with an inspection history were defined as properties that had been inspected in two or more years from 2017 to 2019, regardless of actual mosquito status.

The house index (HI), the percentage of houses with larvae or pupae, was calculated for each year and for the overall time period. The association between inspection history and property status was analyzed at the 5% level of significance using Pearson's Chi square test with Yates' continuity correction. Past and most recent property status were analyzed at the 5% level of significance using McNemar's test. Data and statistical analysis were conducted using R Studio Pro Version 1.2.5019-6 and two-tailed p-values <0.05 were considered significant.

RESULTS AND DISCUSSION

The house index was 19.54% in 2017, 22.83% in 2018, 25.08% in 2019, and 23.9% overall from 2017 to 2019 (Poster, Table 1). *Aedes aegypti* had been re-detected in the District in 2017, after a two-year absence, which triggered door-to-door yard inspections of 261 properties in the infested area. In 2018, 8.7% (N=552) of properties had an inspection history compared to 12.6% (N=1,619) of properties in 2019 (Poster Table 1).

For properties inspected in 2018, mosquito breeding was found on 35.4% of properties with an inspection history, compared to 21.6% of properties that had never been inspected (Poster Table 2, df=1, P=0.046). The 2019 inspection data included properties that had been inspected in 2017 and 2019, with a one-year gap between inspections, as well as properties that had been inspected sequentially in 2018 and 2019 or in all three years. When examining the association between property status and inspection history, larvae or pupae were found on 25.8% properties that had not been inspected in a previous year compared to 18.6% of properties with a sequential inspection history (Poster

Table 1.—House indices and inspection history for DVCD property inspections from 2017 to 2019.

Year	N	House Index (%)	95% CI (Lower-Upper)	Inspection History (%)
2017	261	19.54	15.17 - 24.80	0
2018	552	22.83	19.51 - 26.51	8.7
2019	1,619	25.08	23.03 - 25.71	12.6
2017-2019	2,420	23.97	22.31 - 25.71	9.9

Table 2.—Property status of inspected properties in 2018 with inspection history from 2017. Inspection history was associated with breeding (P=0.04603).

INSPECTION HISTORY	PROPERTY STATUS		
	Breeding	Not Breeding	TOTAL
Yes	17	31	48
No	109	395	504
TOTAL	126	426	552

Table 3.—Property status of inspected properties in 2019 with sequential inspection history from 2018. Breeding was associated with not having an inspection history (P=0.03598).

INSPECTION HISTORY	PROPERTY STATUS		
	Breeding	Not Breeding	TOTAL
Yes	36	158	194
No	365	1,050	1,415
TOTAL	401	1,208	1,609

Table 4.—Property status of inspected properties in 2019 with gap inspection history from 2017. Breeding was not associated with inspection history (P=0.0952).

INSPECTION HISTORY	PROPERTY STATUS		
	Breeding	Not Breeding	TOTAL
Yes	41	163	204
No	365	1,050	1,415
TOTAL	406	1,213	1,619

Table 5.—Prior property status compared to the latest property status recorded in 2018 or 2019 among properties with an inspection history from 2017 or 2018. Prior breeding is associated with breeding in subsequent mosquito seasons ($P<0.001$).

PRIOR PROPERTY STATUS	LATEST PROPERTY STATUS		
	Breeding	Not Breeding	TOTAL
Breeding	33	67	100
Not Breeding	18	122	140
TOTAL	51	189	240

Table 3, df=1, P=0.036). Property status was not associated with inspection history when properties that were inspected only in 2017 and 2019 were included in the analysis (Poster Table 4, df=1, P=0.095). Among properties with an inspection history, having a gap year was significantly associated with future breeding (P=0.03). This may indicate that any knowledge gained from inspections does not last more than a year without reinforcement.

There was a positive association between inspection history, regardless of immature detection, and property status in the 2018 mosquito season, but a negative association in 2019. This difference may reflect the change in distribution of *Ae. aegypti* from a predominantly lush habitat, with abundant sources and high-water usage, to more varied environments across the District. Changes in tools and education methods that began in 2018 and a greater awareness of invasive *Aedes* among District residents in 2019 also could have contributed to this difference (Poster Fig. 1).

When examining the association between prior property status and the latest inspection results, immatures were found at 33% of properties that had prior immatures compared to 12.9% of those with none (Poster Table 5, df=1, $P<0.001$). Continued immature presence across multiple years could be the result of re-infestation from cryptic sources combined with persistent resident behaviors that favor mosquitoes.

CONCLUSION

Although properties with a history of mosquito detection were more likely to continue producing in subsequent years, it was unlikely for households where immatures were not found to start producing mosquitoes. This difference likely represents poor source management habits on the part of the residents combined with the existence of cryptic sources that allow for re-infestation. Focusing control efforts based on past property status may be a more efficient means to manage areas with known *Ae. aegypti* infestations.

The inspection process, regardless of the presence or absence of *Ae. aegypti,* has mixed results in preventing mosquitoes depending on the year of the inspection and whether the property was inspected in sequential years or not. This may be the result of changes in inspection protocols and educational materials as well as differences in the distribution of the mosquito. Resident education needs to be improved, perhaps with the addition of annual reminders for those with a history of mosquito production to reinforce lessons learned during inspections.

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First live-action drone workshop at MVCAC 2020 Annual Conference

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MVCAC held its first live action drone workshop during the 2020 Annual Conference. Over 40 mosquito control experts from 18 Special Districts attended to observe two treatment and two surveillance drones at work (Figure 1A). Changes in regulations regarding the commercial use of drones, otherwise known as unoccupied aircraft systems (UAS), now afford mosquito control agencies the opportunity to use UAS for aerial inspections of the landscape and to apply pesticides (FAA 2016, Buettner et al. 2018). The environment can surveyed with UAS for accumulated surface water where mosquito larvae have the potential to grow (Hardy et al. 2017, Suduwella et al. 2017), and with high powered zoom cameras, mosquito larvae can be visualized with a UAS from tens of feet above the water surface (Haas-Stapleton et al. 2019). Heavy-lifting UAS can carry a large quantity of pesticide and be programed for autonomous flight and high precision pesticide applications. Workers using UAS for mosquito control in environmentally sensitive or hazardous areas (e.g., wildlife refuge or wastewater treatment plant, respectively) can do so without damaging the habitat or risking injury. Reduction in injury risk alone may alone justify intensive use of UAS for mosquito control as doing so may substantially reduce worker injury rates and workers' compensation or insurance claims.

Scott Schon, Lead Vector Control Technician from Placer MVCD, demonstrated the liquid application capabilities of their DJI Agras MG-1S drone (Figure 1B) while Bill Reynolds, CEO at Leading Edge Aerial Technologies, showed granular applications using their PrecisionVision 35 drone (Figure 1C). Tom McMahon, Vector Biologist with Alameda County MAD, demonstrated the visualization capabilities of their DJI Mavic 2 Enterprise Zoom. Eric Haas-Stapleton, Laboratory Director at Alameda County MAD, demonstrated the capabilities of their DJI Matrice 210 RTK for inspecting the land surfaces to workshop participants (Figure 1D) who viewed the flight imagery in real time using a custom bank of Epson Moverio FPV Smart Glasses that were kindly provided by Werner von Stein from the SF Drone School Research Center (Figure 1E).

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Figure 1.—The first UAS workshop at the 2020 Annual Conference of MVCAC. (A) Over 40 participants from 18 Special Districts participated in the workshop. (B) Scott Schon from Placer MVCD readying their DJI Agras MG-1S for a liquid application. (C) Bill Reynolds from Leading Edge Aerial Technologies piloting their PrecisionVision 35 UAS to apply granular product. (D) Eric Haas-Stapleton from Alameda County MAD preparing their DJI Matrice 210 RTK to search for surface water from aloft. (E) Workshop participants viewing the real time imagery from the Matrice 210 RTK in flight using custom bank of Epson Moverio FPV Smart Glasses that were kindly loaned to the workshop by Werner von Stein from the SF Drone School Research Center. Photo credits: A, B, C, D by Werner von Stein from the SF Drone School Research Center; E by Piper Kimball from Leading Edge.