ASSESSMENT OF INTEGRATED MOSQUITO MANAGEMENT AND ECOLOGY OF *CULEX* MOSQUITOES IN THE URBAN SOUTHWEST

by

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Abstract

Culex species mosquitoes are responsible for the transmission of West Nile virus (WNV) and other encephalitic viruses within the United States. In Arizona, *Culex quinquefasciatus* and *Culex tarsalis* are the principal vectors of WNV and St. Louis encephalitis (SLEV). This research examines the desert ecology of these mosquitoes in the context of vector control. Specifically, the study examines how human land-use and vector control activities influence both mosquito abundance and WNV and SLEV presence. Chapter 1 investigates the efficacy of larvicide applications to public drainage features. Chapter 2 analyzes the relationship between land-use and female mosquito density and virus presence. Chapter 3 assesses the susceptibility of *Cx. quinquefasciatus* adults to the insecticides commonly used for WNV prevention in Maricopa County, Arizona which is home to the Phoenix metropolitan area. We found that dry well drainage features are an important habitat for *Cx. quinquefasciatus* mosquitoes, but standard larvicide application techniques have limited efficacy due to the water depth. Suspending either of the larvicides Altosid or *Bti/Ls* at the same water level as the mosquito immatures caused significant reduction on viable larvae. There are many significant associations in adult *Cx. quinquefasciatus* and *Cx. tarsalis* density with designated land use throughout the greater Phoenix area. Notably, land areas designated as agriculture and active open space were positively associated with mosquito density, while single and multi-family residential areas, golf courses, and vacant space were negatively associated. The odds of finding virus-infected mosquito pools were higher residential areas, agriculture, and active open space land use categories and lower in industrial, commercial, and business areas, golf courses, inactive open space areas, and vacant areas. Pyrethroid class adulticides have been used exclusively in Maricopa County for mosquito control purposes. Mosquito immatures were collected from six

different 1-mile square blocks within the cities of Chandler and Gilbert, Arizona, near Phoenix. Immatures were raised until adults and then tested using the CDC bottle bioassay against active ingredients in insecticides used for mosquito control by Maricopa County. All mosquitoes demonstrated exceedingly high levels of resistance to all insecticides tested. The research in this dissertation provides meaningful data on *Culex* mosquito ecology and control in the desert southwest. This information can be used by vector control agencies to improve mosquito surveillance and control.

Chapter 1 - Effective larval sampling and treatment of urban drainage features for mosquito vector control in Maricopa County, Arizona

Abstract

Culex quinquefasciatus mosquitoes are responsible for endemic transmission of West Nile virus (WNV) and St. Louis encephalitis virus (SLEV) within the state of Arizona. *Cx. quinquefasciatus* utilize a variety of water holding sources to complete their immature life cycle in. Maricopa County Vector Control (MCVC) treats urban drainage features with larvicides to reduce this important *Cx. quinquefasciatus* habitat. This study looked at the efficacy of the treatments conducted by MCVC, and subsequent modified treatments completed by the University of Arizona on dry well drainage features within the cities of Chandler and Gilbert, Arizona. We found that sinking larvicide briquets do not impact larval presence and that larvicide needs to be located at the surface of the water where the immatures are present. We also found that FourStar *Bti* briquets were not an effective larvicide and were associated with positive odds of finding viable larvae. Altosid Methoprene and VectoMax FG *Bti*/*Ls* products were both effective at reducing the odds of finding viable immatures within dry wells if they were applied to and kept at the water surface, with Altosid being the most effective. The results of this research can be used in larval control larvicide programs to increase the efficacy of mosquito population suppression.

Introduction

Over 3000 mosquito species are found throughout the world, with hundreds of species being vectors of diseases such as malaria, dengue, and encephalitis viruses. Arizona is home to 46 species of which 13 are recognized vectors. West Nile virus and St. Louis encephalitis are the most common arboviruses transmitted within the state. Dengue virus, however, was recently found in Maricopa County in late 2022 (Maricopa County, 2023).

Most mosquitoes spend their adult lives within a close distance of their larval habitat (Verdonschot & Besse-Lototskaya, 2014). All mosquitoes require water for their aquatic larval and pupal development and will utilize a wide assortment of water sources that are within range of their preferred hosts. Understanding and identifying the potential habitats in human populated areas is the first step in controlling both the vectors and the spread of vector-borne disease.

Mosquito larval habitat

Larval habitats vary between species and can be categorized into three types: running water, permanent still water, and transient water sources. Fast moving water is not commonly used for larval development as increased water speed can flush larvae away. Maintaining themselves within that environment involves a high energy cost. Species that do use moving water tend to reside near vegetation along the bank and away from the main water course (Amadi et al, 2018; Nikookar et al., 2017; Rejmánková et al., 2013; Vanek et al., 2006). Permanent or semi-permanent still water sources maintain water retention for extended periods of time and sustain aquatic vegetation. These sources include freshwater ponds, brackish water, and polluted water holding areas (Amadi et al, 2018; Burke et al., 2010; Nikookar et al., 2017; Reiter & LaPointe, 2009; Rejmánková et al., 2013; Vanek et al., 2006). Mosquitoes that prefer these water sources normally lay their eggs directly on the water surface and the eggs cannot resist desiccation. Larvae tend to reside within vegetated areas, protected pockets, or other areas that provide shelter from open water difficulties. Transient water sources include woodland pools, retention basins, and floodwater areas, as well as natural and artificial containers such as tree holes, small water holding-plants, public drainage structures, flowerpot saucers, plastic tubs, and tires. Water within these sources is usually caused by rain or snowmelt and is only present for a limited amount of time. Many of these water sources are anthropogenic, which contributes to the overall range expansion of species, and allows human inhabited areas to sustain mosquito populations. Some container-breeding species that can lay desiccation resistant eggs will utilize these sources due to lifecycles requiring both wet and dry periods (Amadi et al, 2018; Buhler et al., 2019, Burke et al., 2010; Nikookar et al., 2017; Rejmánková et al., 2013; Tinker, 1964; Vanek et al., 2006; Walker et al., 2018). While some mosquito species have narrow preferences for larval development, other species will utilize a wide range of water sources.

Mosquito larvae are suspension feeding insects that ingest fine particulate organic matter from their environment (Merritt et al., 1996). They have an inverted orientation keeping their heads down from the water surface and siphon tube at the surface of the water for oxygen intake. When feeding in areas with low food levels, immatures will dive down into the habitat to locate potential food sources (Merritt et al., 1992). Diving behavior can also be triggered by water surface disruption or light/shadow stimuli as a way to avoid potential predation (Workman & Walton, 2003).

Important urban southwest mosquito vectors, *Culex quinquefasciatus* (Say) (Diptera: Culicidae) *Culex tarsalis* (Coquillett) (Diptera: Culicidae), and *Aedes aegypti* (Linnaeus) (Diptera: Culicidae), complete their lifecycle within the direct proximity of humans using a variety of man-made or natural water holding containers or environments for larval development (Burke et al., 2010; Christophers,1960; Ponlawat & Harrington, 2005; Scott et al., 2000; Walker et al., 2018). Ae. aegypti lay drought-resistant eggs at water level on the sides of containers (Russell et al., 2001; Trpiš, 1972) and if the water level decreases or is removed, the eggs can remain viable until water returns to the container and contacts the eggs, triggering them to hatch (Russell et al., 2001). The viability of the eggs decreases as time increases with a 90% viability occurring at two months and a 50% viability occurring at 4 months (Soares-Pinheiro et al., 2016). *Cx. quinquefasciatus* and *Cx. tarsalis* eggs are laid directly on the water surface in rafts and hatch quickly. *Culex* mosquitoes regularly utilize larger sources of water such as permanent and semi-permanent water bodies as well as transient water sources for larval development (Burke et al., 2010; Reiter & LaPointe, 2009). As temperatures rise, larvae complete their development faster, reducing the time the insect requires a water source (Huang et al., 2019). This is especially important in Arizona where high temperatures can reduce development time, decreasing the number of days a water source needs to be available.

Drainage structures as mosquito larval habitat

Drainage structures and other subterranean water sources can provide an adequate environment for mosquito larvae to thrive. While the makeup and construction of each structure varies, there are normally areas within each that retain water long enough for larval development to occur (Arana-Guardia et al., 2014; Hribar et al., 2004; Kay et al., 2002; Kwan et al., 2008; Rey et al., 2006; Smith & Shisler, 1981; Su et al., 2003). Drainage structures and subterranean water sources have been found to be a significant habitat for both *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes in the United States, Mexico, Colombia, Australia, and the Caribbean. (Arana-Guardia et al., 2014; Kay et al., 2000; Manrique-Saide et al., 2012; Montgomery et al, 2004; Paploski et al., 2016; Popko et al., 2018). However, the majority of the structures sampled had a depth of under 4 feet (1.21 meters) and the studies do not distinguish how close the water level was in relation to the surface for each species that was collected.

Urban drainage systems in the desert southwest of the United States typically do not contain the intricate and large storm drain networks that are common in wetter regions of the country. The drainage features present in the Phoenix metropolitan area are designed to collect and absorb water within each 1-mile square (1.61 kilometer square) block. This requires the utilization of multiple types of drainage features that lead the water to the lowest points in the watershed, usually a wash, greenbelt, park, or retention area. The drainage feature at the end of the watercourse in greenbelts, parks, and retention areas is called a dry well. Dry wells are underground vertical water structures that dispose of unwanted runoff or stormwater. Most dry wells are cement lined cylinders with an average diameter of 3.92 feet (1.19 meters) and depths ranging from 2 feet (0.61 meters) to 85 feet (25.91 meters) (Edwards et al., 2016). They normally have a gravel bottom and a slotted maintenance hole cover at the surface. Dry wells differ from storm drain collection reservoirs in that there are no discharge pipes that transfer water from the dry well to a larger storm drain network. The water instead stays within the dry well until it either evaporates or permeates into the groundwater through infiltration (Edwards et al., 2016). Much of the research on subterranean larval presence focuses on storm drainage systems or septic tanks which do not have the same structure or dimensions as a dry well. Dry wells should be studied separately to understand their unique characteristics in terms of habitat for immature mosquitoes. Many counties in Arizona utilize dry wells as a component of water management but most do not have robust vector surveillance or control programs.

Mosquito larval control

Controlling the mosquito population at the larval phase is ideal as immatures are isolated in a water source that can be directly treated, affecting all immatures at once. For transient water sources, removing the aquatic habitats through drainage of water bodies or removal of water-holding containers can be highly effective if the majority of larval habitats can be identified and removed (Gubler & Clark, 1996; Leontsini et al., 1993; Walker & Lynch, 2007). If removal of the source is not possible, treatment options include physical barriers that restrict mosquito access to the water source, biological agents such as fish and plants that consume immature mosquitoes, non-insecticide chemicals such as oils that coat the water surface and inhibit air intake through the air siphons of the larvae and pupae (Couret et al., 2020; Floore, 2006; Homski et al., 1994; Nathan et al., 1996), or larvicides. treating water with a larvicide can either kill the larvae upon consumption as provided with agents such as *Lysinibacillus sphaericus* (*Ls*) (previously known as *Bacillus sphaericus* (*Bs*) or *Bacillus thuringiensis* subspecies *israelensis* (*Bti*) or inhibit larval growth as with active ingredients like S-Methoprene (Loke et al., 2010; Marcombe et al., 2011; Ritchie et al., 2010). The larvicidal activity in *Ls* is attributed to two polypeptide protein protoxins and the activity in *Bti* is attributed to four major and two minor polypeptide protein protoxins. Due to the number of peptides in *Bti*, resistance has not been discovered, however, resistance to *Ls* has been documented in several *Culex* mosquito populations (Rao et al., 1995; Su et al., 2019; Yuan et al, 2000). By combining specific *Ls* and *Bti* toxins together, studies have found an increased efficacy and less resistance compared to the wild type strains alone (Lacey et al., 2007; Wirth et al., 2010). Aside from resistance, many larval and environmental factors can impact the efficacy of *Ls* and *Bti* including feeding rates, larval density, water temperature, UV exposure, the organic content of the water, toxin content of the larvicide, and larvicide saturation at the target location (Aly et al., 1988; Beck et al., 1996; Lacey et al., 2007; Mulla et al., 1990; Mulligan III, et al., 1980; Nayar et al., 1999; Nguyen et al., 1999; Wraight et al., 1987).

Maricopa County Vector Control (MCVC) has an extensive mosquito control program that currently includes over 800 CO2-baited adult mosquito surveillance traps, each within a 1-mile square block throughout the greater Phoenix area. MCVC uses the traps to monitor mosquito and arbovirus activity and maintains specific thresholds that trigger adult mosquito insecticide spraying events. In addition to adulticide treatments, MCVC also treats urban drainage features within trapping blocks with larvicides that target mosquito immatures. Blanket treatments have occurred at the beginning of the mosquito season and are composed of long-lasting larvicide applications to public drainage features that are visible from city streets. However, most MCVC immature treatments are focused on treating known habitat or problem areas. MCVC has historically utilized several types of larvicides including FourStar *Bti* and Altosid briquets (Central Life Sciences, Council Bluffs, IA), and VectoBac and VectoMax granules (Valent Biosciences, Libertyville, IL). The University of Arizona partnered with MCVC to assess vector control impacts of applying long-lasting larvicide formulations to drainage features.

Methods

Study site

Chandler and Gilbert are cities within the greater Phoenix metropolitan area, in Maricopa County, Arizona. Both cities are located southeast of the Phoenix city center (Figure 1). According to the 2020 Census, Chandler had a population of 275,987 and Gilbert had a population of 267,918 (US Census Bureau, 2022). The climate in Maricopa County is a sub-tropical desert type with low annual rainfall (6.82 inches average over the past 10 years) and low relative humidity (44% average). Daytime temperatures averaged 93.4°F throughout the summer months over the past 10 years (National Weather Service, 2023) with monsoon rainfall occurring from July through October.

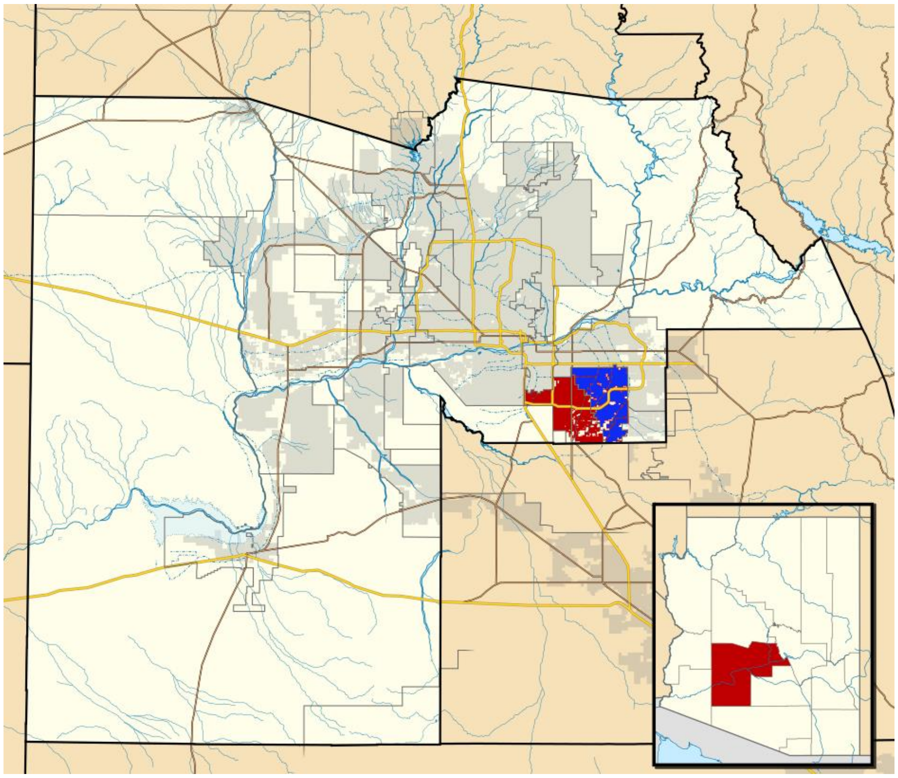

Figure 1: The Cities of Chandler (Red) and Gilbert (Blue) within Maricopa County, Arizona.

The study identified twenty, 1-mile square blocks, 10 in the city of Chandler and 10 in the city of Gilbert, with established mosquito populations. The original target species was Ae. aegypti, but assessments of *Culex* spp. were also included. In each city, nearby or adjacent study blocks were paired based on similar demographics (Figure 2). In each block, a five zone quadrant was overlaid on the 1-mile square block and a dry well within each quadrant was selected as the sample site in order to better represent the entire block (Figure 3). Larval sampling was focused on dry wells due to them being the most likely structure to hold water and harbor mosquito immatures. In each block, dry wells were sampled biweekly to assess efficacy of long-lasting larvicides during the peak of mosquito activity in July through October (the rainy season when mosquitoes are most active) in 2017 through 2020. Sampling was conducted using an inline hand pump that drew water from the dry well and deposited it into a bucket at the surface (Figure 4). The intake hose was 20 feet long in order to access water levels far into the dry well. The funnel at the end of the intake hose assisted in channeling the larvae into the intake hose. Two liters of water was removed from each dry well and inspected for the presence of immatures. If matures were found, they were transported back to the University of Arizona to be identified.

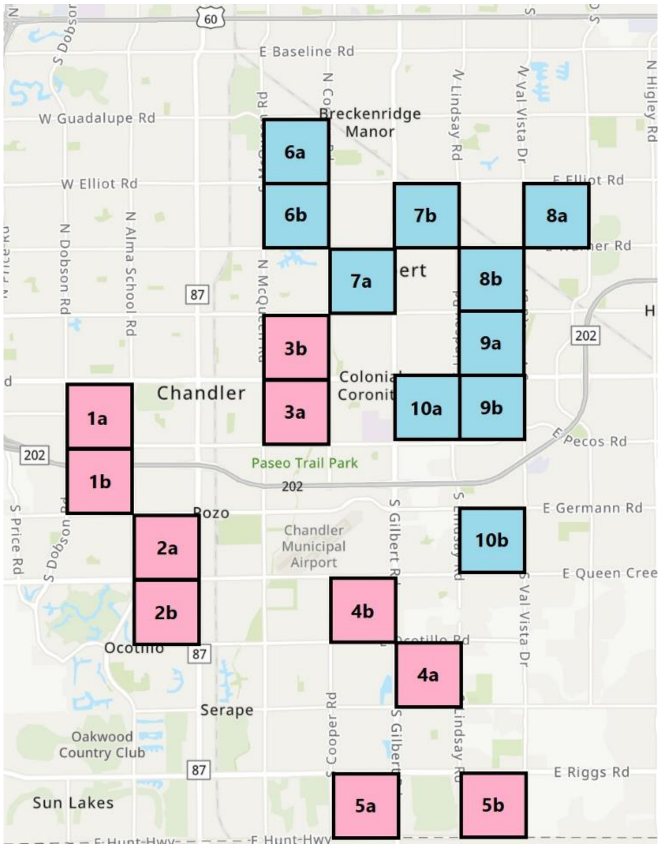

Figure 2: Paired study sites. Red sites represent the city of Chandler and blue sites represent the city of Gilbert. Site a represent treatment sites, and site b represent control sites. Each site is 1-mile square.

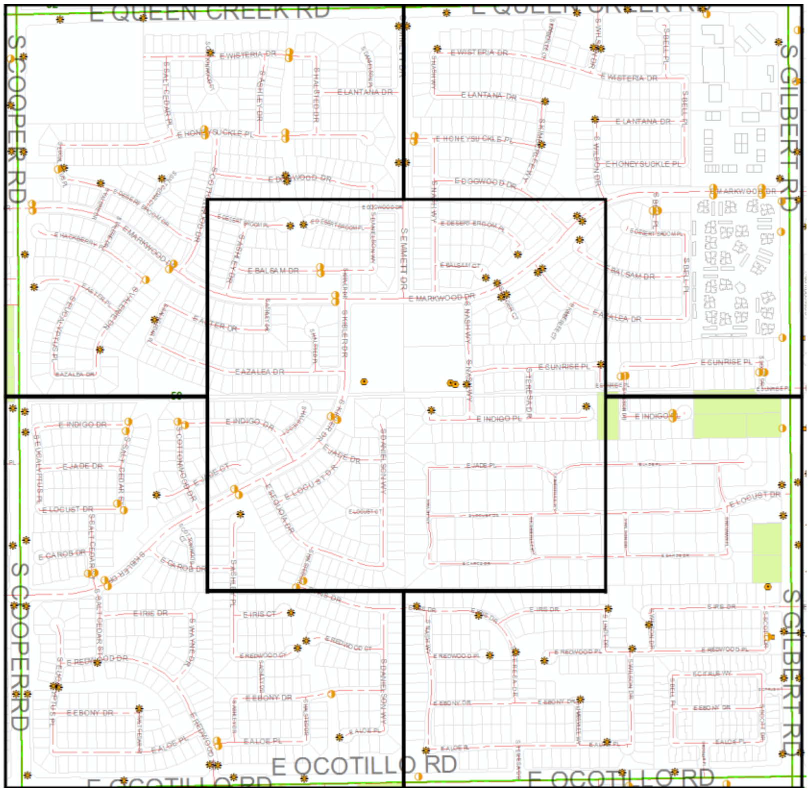

Figure 3: Example of a 1-mile square study block quadrant with known drainage feature locations. One dry well was selected in each quadrant.

Figure 4: Larval siphon pump.

2017

For each pair of 1-mile square blocks, one block was randomly selected to be the treatment block while the other was the untreated control. In the treatment blocks, all public drainage features were to be treated by MCVC with VectoBac G *Bti* granules. Five dry wells in each block were sampled biweekly for the presence of mosquito immatures (a total of 100 dry wells; 50 treated and 50 control).

2018

The city of Gilbert was removed from the larval study due to a lack of immature habitat in public drainage features. The ten Chandler study blocks were included in the larval control study using the previously established dry well sampling sites (50 dry wells total; 25 treated and 25 control). In 2017, it was discovered at the end of the season that some of the sampled dry wells in treated blocks did not receive larvicide treatments by MCVC. To ensure larvicide application of drainage features within treatment blocks, all accessible public and private drainage features within the five Chandler treatment blocks were treated by University of Arizona personnel with 180-day FourStar *Bti* briquets. FourStar 180 day briquets contain 6% Ls, and 1% *Bti* as active ingredients per dose (1 briquet) (Central Life Sciences, 2013). It was difficult to quantify the number of briquets that were needed to treat a dry well due to the differences in dry well volume and the inability to see the bottom of some dry wells due to them holding water. Treatment began with one briquet in each dry well. Treatment was conducted the second week of July and sampling began two weeks later.

After two sampling weeks with no reduction of viable larvae in dry wells, the decision to modify the treatment was made. Instead of only FourStar briquets, 150-day Altosid XR briquets were added to two of the previously treated blocks. Altosid XR 150 day briquets contain 2.1% Methoprene as an active ingredient per dose (1 briquet) (Central Life Sciences, 2010). The remaining three blocks received two additional 180-day FourStar *Bti* briquets in each dry well. Dry wells were then sampled for the presence of mosquito immatures bi-weekly beginning the first week of September. Larvae found in an Altosid treatment area were transported to the University of Arizona to be observed for possible pupal eclosion.

2019

Twelve dry wells in Chandler previously identified to hold water and support mosquito immatures within study blocks were randomly assigned a treatment type and were treated by the University of Arizona with either 150-day Altosid XR briquets or 180-day FourStar *Bti* briquets (Figure 5). Larvicide briquets were positioned within a floating device designed to keep the briquets at the same level as the immatures despite fluctuations in water volume (Figure 6). The device was made from minimal components so that application could be made without the removal of the maintenance hole cover, and to keep from adding to excessive debris within the dry well. During each sampling event, the device was visually identified to be present. A total of four dry wells were treated with Altosid, four were treated with *Bti*, and four were left as control sites. The twelve dry wells were then sampled weekly between July and October for the presence of mosquito immatures. If larvae were found in a dry well treated with Altosid, they were transported to the University of Arizona to be observed for possible pupal eclosion.

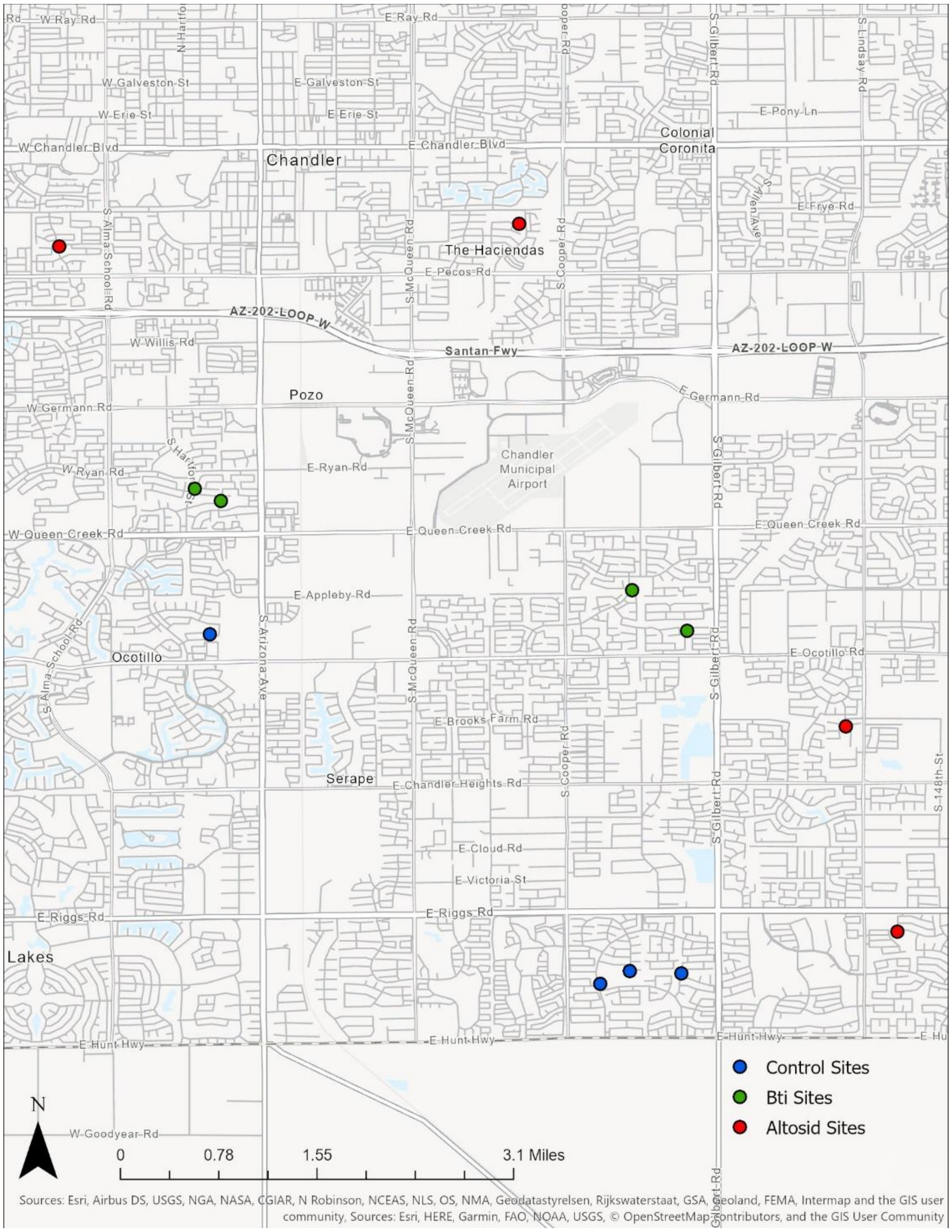

Figure 5: 2019 and 2020 treatment block locations.

Figure 6: Float device containing a FourStar Bti briquet designed to keep the larvicide near the water level in which the larvae reside.

2020

The experimental design used in 2019 was repeated but VectoMax FG *Bti* granules were used in place of the 180-day FourStar *Bti* briquets. VectoMax FG contains 2.7% Ls, and 4.5% *Bti* as active ingredients per dose (115g) (Valent Biosciences, 2015). Dose calculations were based off of a 40 foot (12.19 meters) deep and 4 foot (1.21 meters) diameter dry well volume of water.

VectoMax FG granules are designed to float, but are loose, so to keep them from being washed away they were kept in a floating muslin bag. During each sampling event, the muslin bag was visually identified to be present. VectoMax FG granules only have a 28-day efficacy window, so the larvicide was replenished accordingly. Weekly sampling of the sites were conducted from August through October.

Analysis

Due to many treatment sites being overlooked for treatment in 2017, we removed the year from final analysis. For the 2018 analysis, only data that was collected after the modified treatment in September were used.

The probability that a site had viable larval presence was modeled with a Generalized Linear Model (GLM) using the logit function. This can be written as,

$$\text{Logit}(p) = \beta_0*(A) + \beta_1*(B) + \beta_2*(C)$$

where A, B, and C are indicators of the treatment type, and β_0, β_1, and β_2 are the log of odds of the probability that the sites with larvicide treatment contained viable larvae. C is the control, or comparison group in this equation. However, there is a potential blocking factor due to a correlation of larval presence within sites that needs to be accounted for. To do this we used a General Estimating Equations (GEE) model to incorporate other elements needed to account for this variation. Previous studies have used GEE in mosquito control analysis (Fillinger et al., 2009; Otieno et al., 2013) and allows researchers to estimate parameters of a generalized linear model with a potential unknown correlation between outcomes. For this study, the collected data and errors could be correlated within each site or time point. The GEE uses a repeated measures analysis for each site and the times in which each site was sampled are modeled to be correlated. Time points that are closer together have a higher likelihood or correlation compared to those that are further apart. Dunnett's test was used to compare the control group with the treatment groups. SAS OnDemand for Academics (2023) was used for all analysis.

Results

The proportion of dry wells that contained suitable habitat for immatures over all sampling events is detailed in Table 1. In 2017, only 5 of the 50 dry wells sampled in the city of Gilbert held water at least once, and only one dry well held water 50% of the time. Due to the limited larval habitat provided by these dry wells (which were functioning properly), Gilbert was removed from sampling after 2017. In Chandler, the proportion of dry wells that held water at least once was 0.48 in 2017 and 0.52 in 2018. The proportion of drywells that held water at least 50% of the time were 0.16 in 2017 and 0.32 in 2018. The experiment changed to focus on just 12 dry wells in 2019 and 2020. All these dry wells contained water at least once in 2019 and 0.92 contained water at least once in 2020. The number of dry wells that contained water at least 50% of the time were 0.75 in 2019 and 0.50 in 2020.

	Proportion of Sites with Larval Habitat Over Sampling Period				
	At least Once	25% of the Time	50% of the Time	75% of the Time	100% of the Time
2017 Gilbert	0.10	0.04	0.02	0.02	0.02
2017 Chandler	0.48	0.34	0.16	0.02	0.00
2018 Chandler	0.52	0.38	0.32	0.16	0.12
2019 Chandler	1.00	0.92	0.75	0.42	0.00
2020 Chandler	0.92	0.75	0.50	0.33	0.25

Table 1: *Proportion of sites with larval habitat over the sampling period*

Ae. aegypti immatures were only found during 2018 and 2019 at very low levels. The overall proportion of species found in dry wells was 0.04 *Ae. aegypti* and 0.96 *Culex* spp. All *Culex* larvae that were identified under the microscope were *Cx. quinquefasciatus*.

The 20 foot intake hose on the pump was adequate to sample from all water holding dry wells. There was variation in the depth of water between dry wells and between sampling dates. There were some sites that barely had enough water at the bottom to siphon from, and there were sites that had water above the maintenance hole cover. Viable *Culex* larvae was taken from dry wells with water over 15 feet below the surface, but *Ae. aegypti* larvae were only found in dry wells with water levels within a few feet of the surface.

For the analysis looking at larvicide efficacy, the estimates for each treatment type by year are located in Table 2. The log-odds are significant for 2019 and 2020, but not for 2018. Log-odds show an increased odds of viable larvae for FourStar *Bti* and a decreased odds of viable larvae for Altosid in 2019. In 2020, there were decreased odds of finding viable larvae in both Altosid and VectoMax FG treated wells. In both years, there were increased odds of viable larvae in control dry wells. The Dunnett's test for the comparison between treatment groups and the control group was not significant in 2018 but was significant for both treatment types and the control sites in 2019 and 2020 (Table 3). In 2019 and 2020, the mean probability that a site had viable larvae was reduced in treatment sites compared to the control site (Table 3). Altosid treatment efficacy varied between 2019 and 2020 (mean 0.20 and 0.07, respectively) but were more effective than either the *Bti* or *Bti/Ls* products. FourStar *Bti* treatments in 2019 (mean 0.70) were not as effective as VectoMax FG *Bti/Ls* treatments in 2020 (mean 0.36). The mean for the probability that a control site had viable larvae was 0.94 and 0.80 for 2019 and 2020, respectively.

Parameter Estimates for Response Model with Empirical Standard Error Estimates						
			95% Confidence Limits			
	Estimate	Standard Error	Lower	Upper	z Value	Pr > |z|
2018						
Altosid	0.151	0.478	-0.786	1.088	0.320	0.752
Four Star *Bti* Briquette	1.219	0.632	-0.019	2.458	1.930	0.054
Control	-0.092	0.546	-1.162	0.978	-0.170	0.866
2019						
Altosid	-1.404	0.468	-2.320	-0.487	-3.000	**0.003**
Four Star *Bti* Briquette	0.837	0.360	0.133	1.542	2.330	**0.020**
Control	2.812	0.583	1.669	3.955	4.820	**<0.001**
2020						
Altosid	-2.587	0.999	-4.545	-0.628	-2.590	**0.010**
VectoMax FG *Bti*	-0.597	0.114	-0.821	-0.373	-5.220	**0.000**
Control	1.399	0.542	0.337	2.461	2.580	**0.010**

Table 2: Parameter estimates for response model with empirical standard error estimates.

Differences of Treatment Least Squares Means Adjustment for Multiple Comparisons: Dunnett				
		Estimate	Standard Error	Adj P
2018				
Altosid	Control	0.243	0.726	0.922
Four Star *Bti* Briquette	Control	1.311	0.835	0.203
2019				
Altosid	Control	-4.216	0.747	**<0.001**
Four Star *Bti* Briquette	Control	-1.975	0.685	**0.007**
2020				
Altosid	Control	-3.986	1.137	**0.001**
VectoMax FG *Bti*	Control	-1.996	0.554	**0.001**

Table 3: Differences of treatment least squares means adjustment for multiple comparisons.

Probabilities of Viable Larval Presence				
	Mean	Standard Error of Mean	Lower Mean	Upper Mean
2019				
Altosid	0.197	0.074	0.089	0.381
FourStar *Bti* Briquet	0.698	0.076	0.533	0.824
Control	0.943	0.031	0.842	0.981
2020				
Altosid	0.070	0.065	0.011	0.348
VectoMax FG *Bti/Ls* Granules	0.355	0.026	0.306	0.408
Control	0.802	0.086	0.583	0.921

Table 4: Mean probabilities of viable larvae presence for significant years.

Discussion

The majority of mosquitoes removed from dry wells were *Cx. quinquefasciatus*. The few dry wells that contained *Ae. aegypti* typically had water levels at or close to the surface. These findings are similar to other studies that have found *Ae. aegypti* utilizing subterranean water sources at shallow depths (Arana-Guardia et al., Kay et al., 2000; Montgomery et al, 2004; Paploski et al., 2016). It is possible that *Ae. aegypti* mosquitoes do not prefer to utilize subterranean oviposition environments unless another factor forces them to abandon preferred oviposition sites. Studies have suggested that utilization of subterranean habitat is linked to reduced container availability at the surface (Chadee & Martinez, 2016; Irving-Bell et al., 1987; Russell et al., 2001) In contrast, *Cx. quinquefasciatus* mosquitoes are known to utilize subterranean water sources much more frequently and at higher numbers (Hazelrigg & Pelsue, 1980; Mackay et al., 2009; Rey et al., 2006; Strickman & Lang, 1986; Su et al., 2003), even deep within mines (Dutta, 1977). In addition, there is evidence of *Culex* mosquitoes maintaining entire populations in a subterranean environment for extended periods of time (Byrne & Nichols, 1999).

The study found differences in dry well drainage between cities. This variability could be due to different dry well design, construction, or upkeep. Dry wells in the city of Gilbert overall drained water properly and did not contribute much to mosquito larval habitat. The city of Chandler had many drywells that failed to drain water promptly and became adequate mosquito larval habitat. In 2017 and 2018 all dry wells sampled that were found to be holding water or full of debris were reported to the city environmental management. Some dry wells were immediately remedied, while others continued to be a habitat for mosquito immatures. These results demonstrate the importance of well-designed and maintained urban drainage in reducing mosquito vector populations.

Sampling dry wells can be challenging for vector control professionals. Standard dip cups do not have the reach to sample low water levels in deep dry wells. Dip cups require the removal of the maintenance hole cover which requires training and specialized tools, and an increase of time at the site. Some dip nets may be malleable enough to make it through the grate slot, but pole length and maneuverability through a grate slot may not be practical. The siphon pump method allows the user to sample the water without removing the maintenance hole cover. Once the flexible funnel is fed through the grate slot, the line can be lowered to the water level. The initial disruption of the water surface may cause the larvae to dive, but the continual uptake of water allows time for the larvae to return to the surface to be funneled into the intake hose. Further assessment of the efficacy of the siphon pump compared to dip cups and nets is being conducted. Just as dry wells were challenging structures to sample for mosquito immatures, they were also difficult to treat effectively with larvicides. The failure of larvicide applications to reduce larval number in treated wells in 2018 was likely due to the large volume of the dry well structures. Mosquito immatures need to reside at the water surface in order to acquire oxygen, so treatment briquets that sink to the bottom of the enclosure did not provide enough saturation of active ingredient in the upper water level where larvae are more active. In theory, the longer that the briquet resides in a specified water body, the higher the concentration of larvicide. However, during monsoon rain events the water in non-draining dry wells is flushed or saturated with new water, driving the larvicide levels down. In addition, when the briquet is resting at the bottom of a dry well, debris, mud, and gravel that is washed into the dry well can easily smother the briquet ceasing the release of the larvicide completely. To address this potential problem, a float device was created that kept the larvicide at the level of the immatures despite the fluctuations in water volume. Floating chlorine dispensers filled with larvicide have been used in larval control but are bulky and require the maintenance hole cover to be removed in order to insert the device. A smaller mesh device made insertion much more convenient and did not require the use of cumbersome tools used to remove maintenance hole covers, but the longevity of the device will not be as effective as a chlorine dispenser.

The analysis model found that Altosid treatments in 2019 and 2020 was effective in reducing viable mosquito immatures in the dry wells. Analysis also showed that VectoMax FG granules could reduce the number of immatures, though not as successfully as Altosid. It was difficult to dose the dry wells precisely due to the fluctuations in water volume and unknown dimensions. The FourStar 180 day briquet specimen label directs that 1 briquet should be used per 100 square feet of surface area. An additional briquet should be added for each additional 100 square feet of water surface, regardless of water depth. In addition, they suggest doubling the application rate when mosquito populations are high, water is heavily polluted, and/or algae are abundant (Central Life Sciences, 2013). The Altosid specimen label directs that 1 briquet should be used per 100 square feet of surface area up to 2 feet in depth. An additional briquet should be used every additional 2 feet of water depth (Central Life Sciences, 2010). VectoMax FG granules are designed to float on the surface of the water, so water depth is not addressed. For storm water or drainage systems, the manufacturer recommends 5-20lbs of granules per acre, or 10-20lbs per acre in areas where very high densities of late instar larvae are present (Valent Biosciences, 2015). Efficacy of the bacterial larvicide products which must be consumed by the target pest may also be influenced by the presence of debris within dry wells. Organic material concentrations can be high in dry wells, especially in park areas with grass that is cut on a regular basis. The debris competes with the larvicide as a food source for larvae and may contribute to the reduction of consumed larvicide.

A limitation of this study is the small sample size of treated dry wells. The initial study was designed with the assumption that larvicides would be effective at suppressing larval development and the intended focus was population effects of widespread larvicidig. In reality, the dry wells were both productive larval habitat but also challenging to treat effectively. Due to this discovery, experimental design changed throughout the years of the study. Ultimately, we were able to apply and compare larvicides on only a small sample of dry wells. The difference in precipitation between years also made it impossible to pool across years. Another limitation to the study was the unsuccessful application of larvicide to study treatment wells. MCVC were initially to treat all dry wells in our treatment blocks in 2017, but we found out later that only drainage features visible from public streets and those that were on public land were treated. In order to address the issue in 2018 the University of Arizona applied larvicide. Initial application was a very large task that took several days to complete with three teams, but we then had to complete it again when we modified the treatment. The length of time needed to treat these blocks reduced the time that we had to conduct sampling events. In contrast, 2019, and 2020 had a much smaller sampling size and while sampling was conducted weekly as opposed to biweekly, the power of the study would have been increased with more sampling locations.

Completing this study in real-world field conditions as opposed to artificially controlled semi-field experiments provided insight into the challenges associated with working with several organizations simultaneously as well as unexpected environmental and structural factors that can influence larval habitat. The findings from this study may provide vector control agencies with information on larval habitat within public drainage features in the desert southwest as well as information regarding the efficacy of larvicide types and methods in how to apply them considering the treatment environment.

Chapter 2 - Assessing the relationship between land-use and both arbovirus presence and mosquito density of *Culex quinquefasciatus* and *Culex tarsalis* in Maricopa County, Arizona

Abstract

West Nile virus (WNV) arrived in Arizona in 2003 and St. Louis encephalitis virus (SLEV) reemerged in 2014. Both diseases have since been endemic and have reached record breaking levels within the past few years. Understanding the ecology of the mosquito hosts and predicting locations of positive mosquito pools are important components to designing effective vector control. Many landscape ecology studies have been completed in the United States examining associations with mosquito density and virus presence, but none have been conducted in the desert southwest. This study analyzes land use within Maricopa County Arizona and its relationship with *Culex quinquefasciatus* and *Culex tarsalis* density and the probability of finding WNV or SLEV positive mosquito pools. We found significantly higher densities of both mosquito species associated with agricultural areas. *Cx. quinquefasciatus* mosquitoes also occur at higher density in active open space areas that include public parks and fields. The relationship between land use and virus presence was less clear. Overall, positive pools were more likely to occur in years with higher mosquito densities. Results from this study suggest vector control professionals should target controls for agricultural and open space areas, especially during years where mosquito density is high.

Introduction

West Nile virus (WNV; Flavivirus, Flaviviridae) and St. Louis encephalitis virus (SLEV; Flavivirus, Flaviviridae) are mosquito transmitted diseases that cause significant human disease burden in the United States, including within the desert southwest. WNV was first isolated in the West Nile district in Uganda in 1937 (Smithburn et al., 1940) and is now the most widely distributed encephalitic flavivirus throughout the world, arriving in Arizona in 2003 (Arizona Department of Health Services, 2023). SLEV was first isolated in 1933 in St. Louis, Missouri, USA (Lumsden, 1958) and the earliest reported cases in Arizona were in 1972 (Diaz et al., 2018). Reemergence occurred between 2011 and 2014 (Ridenour et al., 2021) but low enzootic activity and human cases were recorded until an outbreak in 2015 when 23 confirmed human cases and 1 death occurred (Venkat et al., 2015). Both enzootic and human cases now occur annually within the state.

Birds are the natural reservoirs and amplifiers of both WNV and SLEV. The enzootic mosquito-bird-mosquito transmission cycle maintains the virus in nature and is primarily completed by *Culex* spp. mosquitoes (Campbell et al., 2002). Human hosts are normally considered to be a dead end for viral transmission due to the inability of the viruses to replicate to the infectious viremic threshold (Higgs et al., 2005). While rare, human to human transmission can occur through blood transfusions and the transplantation of tissues, cells, or organs (Kleinschmidt-DeMasters et al., 2004; Pealer et al., 2003; Venkat et al., 2017), however increased screening protocols have greatly reduced this occurrence for WNV (Busch et al., 2019). Donated blood is not normally screened for SLEV at this time.

Passeriformes such as the common house sparrow as well as Columbiformes such as the mourning dove are important amplifying hosts for both WNV and SLEV (Langevin et al., 2005;

Nemeth et al., 2009). In addition, Passeriformes are associated with higher WNV infection rates within densely human populated areas compared to all other orders of birds (Bradley & Altizer, 2007). While mosquito-host transmission is the most important mode of viral spread, other less significant forms of direct transmission between birds can occur including contact with fecal and/or oral discharge, or by the mutual grooming of feathers (McLean et al., 2001). While there are many similarities between WNV and SLEV, the viruses exhibit differences in viremia and virulence characteristics in birds. WNV can cause a high level of viremia leading to the mortality of the host whereas SLEV viremia levels are typically low and are not associated with avian mortality (Maharaj et al., 2018).

Arizona has a vast array of bird species that are either native, invasive, or migratory. Arizona Game and Fish (2023) report that there are 534 documented bird species in the state, 300 of which have been documented as breeding in the state. At least 237 neotropical migrant species have been documented in Arizona, with 163 of them nesting. The majority of migratory birds arrive in the desert lowlands by mid-April and depart in October. Seven non-native species, including the house sparrow, have been documented in the state for many years (Shrey et al., 2011). In 2010 a team from the Centers for Disease Control and Prevention (CDC) collected birds from the east valley in Maricopa County and tested them for WNV (Komar et al., 2013). A total of 300 birds representing 17 species were captured and blood tested. WNV was confirmed in 144 of the samples from 14 species. In addition, mosquitoes were captured, and blood meals were sequenced to determine the host species. The study found that the house sparrow (Passeriformes), house finch (Passeriformes), mourning dove (Columbiformes), and great-tailed grackle (Passeriformes) were frequently infected and are likely important amplifying hosts for WNV within the state. Significantly, the majority of *Cx. quinquefasciatus* blood meals were primarily taken from house sparrows. Similar studies for SLEV have not been conducted in Arizona. House sparrows tend to roost lower to the ground in bushes, shrubs, and in accessible areas on the outside of human dwellings. However, much of the ecology of this important disease reservoir within the desert southwest is not known.

Most cases of human WNV and SLEV transmission occur in the East and Northeast Valley of the greater Phoenix area (Maricopa County, 2017, 2019) which include the cities Chandler, Gilbert, Mesa, Queen Creek, Apache Junction, and Scottsdale. The 2020 Census listed Phoenix, Mesa, Chandler, Gilbert, Glendale, and Scottsdale as the highest populated cities in the county, four of which belong to the East and Northeast Valley. These areas are surrounded by and contain many agricultural fields, as well as properties with grass lawns or community green belt areas that require flood irrigation, and several manufactured lakes and ponds (Maricopa Association of Governments, 2022). Previous research indicated WNV and SLEV could be reintroduced annually to non-endemic areas via migratory birds (Auguste et al., 2009; Hubalek, 2000; Kopp et al., 2013; Rappole et al., 2000; Rappole & Hubálek, 2003; Ungureanu,1999). There is recent evidence, however, that both WNV and SLEV are now endemic to Arizona (Hepp et al., 2018; Ridenour et al., 2021) and may not require reintroduction into the state.

Arizona is home to over forty-five species of mosquitoes, but *Cx. quinquefasciatus* and *Cx. tarsalis* are the most common *Culex* species found throughout the state and the primary vectors of WNV and SLEV. In Maricopa County, the population dynamics of *Cx. quinquefasciatus* and *Cx. tarsalis* differ slightly throughout the year but density for both species is highest March through November. *Cx. quinquefasciatus* has the highest density occurring in August while *Cx. tarsalis* has the highest density in March and April. The density declines for both species in June and July but increases again when the rainy season begins (Wilke et al., 2022).

Culex mosquitoes prefer to host seek after dusk with peak activity one to three hours after sunset during the warmest, driest part of the night (Reisen et al., 1997). Specific roosting behavior can be particularly attractive to *Culex* species mosquitoes. Birds that roost high in canopies, such as the American robin, are significantly less likely to be fed on by mosquitoes at night compared to lower roosting birds, such as the house sparrow (Diuk-Wasser et al., 2010; Janousek et al., 2014; Thiemann et al., 2012). Land use and landscape ecology can be important determinants in the distributions of mosquito species (DeGroote & Sugumaran, 2012). *Cx. quinquefasciatus* have routinely been found to be associated with urban environments (Deichmeister & Telang, 2011; Savage et al., 2014), while *Cx. tarsalis* have been found to prefer rural areas (Chuang et al., 2011; Griffin D., 2008; Larson et al., 2010; Schurich et al., 2014). *Cx. quinquefasciatus* mosquitoes will utilize backyard water holding containers, public drainage features, fountains, and fishponds for oviposition sites (Muller et al., 2010; Santana-Martinez et al., 2017), as well as the larger water sources that *Cx tarsalis* mosquitoes prefer such as agricultural trenches, stagnant swimming pools, wetlands, canals, and water treatment basins (Bhattacharya et al., 2016; Noori et al., 2015; Reisen et al., 1989; Wilke et al., 2022).

Identifying areas that are associated with a higher mosquito density, or areas in which mosquitoes test positive for WNV or SLEV at a higher rate could provide mosquito control professionals with information that could help prioritize resource use within those areas, or preemptively target areas with control strategies to help mitigate disease spread. This study will examine the relationship between type of land use in Maricopa County, Arizona and determine whether *Cx. quinquefasciatus* or *Cx. tarsalis* mosquitoes are associated with certain land use categories over others. The study will also assess whether SLEV or WNV presence are associated with specific land use categories.

Methods

Study Site

Maricopa County, Arizona is the fourth largest county in the United States. It is located in the south center part of the state and has a population of 4,420,568 (US Census Bureau, 2021) which includes the greater Phoenix metropolitan area. The climate for Maricopa County is a sub-tropical desert type with low annual rainfall (6.82 inches average over the past 10 years) and low relative humidity (44% average). Daytime temperatures averaged 93.4°F throughout the summer months over the past 10 years (National Oceanic and Atmospheric Administration, 2023) with monsoon rainfall occurring from July through October.

Maricopa County Vector Control (MCVC) has an extensive mosquito control program that currently includes over 800 CO2-baited surveillance traps, each within a one-mile square block, throughout the greater Phoenix area. MCVC uses the traps to monitor mosquito and arbovirus activity and maintains specific thresholds that trigger adult mosquito insecticide spraying events. The majority of traps are checked on a weekly basis throughout the entire year. However, there are some traps, such as traps placed temporarily in response to complaints, which are not part of routine surveillance and are not surveyed throughout the year.

Data

Land use geodatabases were obtained from the Maricopa Association of Governments. Data for the years 2014, 2016, 2017, 2019, and 2020 were obtained for this study as data is not collected or published annually. Land use category definitions varied as time progressed in that one category may have been broken into multiple categories to increase specificity. For example, in early years open space categories included active open space, inactive open space, and water. In later years open space was further broken down into active open space, passive open space, water, desert parks and preserves, washes, and public land. Individual land use categories were combined for the purpose of this analysis (Table 1). Mosquito trapping data for the corresponding years was obtained from MCVC. Daily surveillance data, by site location, were sorted by mosquito species and were compiled into annual totals. All species besides *Cx. quinquefasciatus* and *Cx. tarsalis* were removed from the dataset. Any site that was trapped under 42 times in the year was removed from the study for standardization purposes. This minimum number of trappings was selected to ensure that the majority of the year in which higher mosquito density is present was included. In 2014, mosquitoes of both species were not tested for SLEV as the outbreak in 2015 initiated routine surveillance. Trap locations within study blocks could vary between years, and the number of trapping locations increased with time. Due to this data were not pooled across multiple years and each year remained independent.

Land Use Categories		
ID	**Name**	**Description**
1	Transportation	Airport, Transportation, Railroads
2	Industrial, Commercial, Business, Public, Other Employment	Tourist Accommodations, Commercial, Industrial, Business Park, Mixed Use, Educational, Medical/Nursing Homes, Religious, Institutional, Public, Special Event, Military, Solar Generating Stations
3	Single Family Residential	All Density Single Family Residential
4	Multi-Family Residential	Multi-Family Residential
5	Agriculture	Agriculture, Orchards, Dairy and Feed Lot
6	Active Open Space	Active Open Space, Parks, Cemeteries
7	Golf Course	Golf Courses
8	Inactive Open Space	Inactive Open Space, Washes, Passive, Restricted Open Space, Undevelopable, Landfill, Proving Grounds, Desert Parks and Preserves, Open Land
9	Water	Water
10	Vacant	Vacant, Abandoned Agriculture, Vacant State Trust
11	Developing	Developing residential and commercial

Table 5: Land use category descriptions

Modeling

All land use geodatabases were uploaded to ArcGIS Pro (version 2.4.2) (Figure1). Annual mosquito trap data .xlsx files were uploaded into the geodatabase using the XY Table to Point tool that created a point feature class based on x, y, and z coordinates from a table. This provided point locations for all MCVC mosquito traps that were utilized during the corresponding year (Figure 2). A literature review was then conducted on the estimated flight range of each species. A mean range of 0.46 miles was determined for *Cx. quinquefasciatus* range based on 14 mark-release-recapture experiments (Lindquist et al., 1967; Macdonald et al., 1968; Medeiros et al., 2017; Reeves et al., 1948; Reisen et al., 1991; Reisen et al., 1992; Self et al., 1971; Verdonschot, 2014). A mean range of 0.41 miles was determined for *Cx. tarsalis* range based on 8 mark-release-recapture experiments (Reeves et al., 1948; Reisen et al., 1991; Reisen et al., 1992; Reisen & Lothrop, 1995; Verdonschot, 2014). The Buffer tool in ArcGIS Pro was used to create a buffer circle in which the radius corresponded to the mean flight range for each species around each trap location point to represent an estimated travel range of the mosquitoes. The Intersect tool, which computes a geometric intersection of input features that overlap and combines them into a single output feature class, was then used to isolate the land use areas that were within each corresponding buffer circle (Figure 3). The Add Geometry Attributes tool was then used to add a new field with the calculated area of each land use polygon within the buffer area. Once complete, an .xlsx file with the new data was created so that it could be manipulated further using MATLAB. Utilizing multiple functions, data clustering was completed and the percentage of each land use category within the flight range of the mosquito for each site location was calculated.

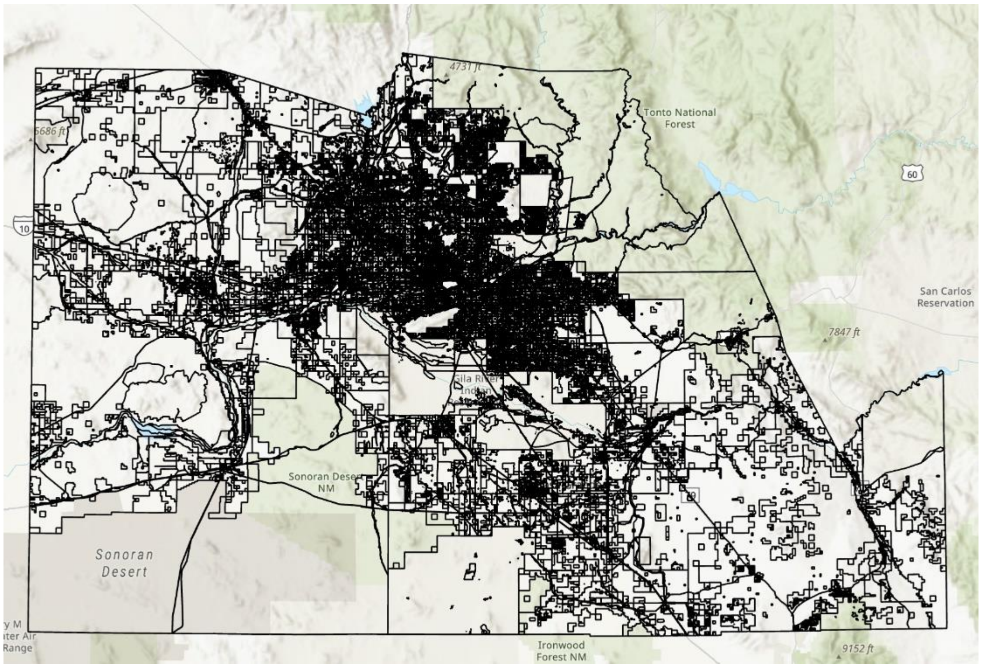

Figure 7: Total Maricopa County land use designation boundaries

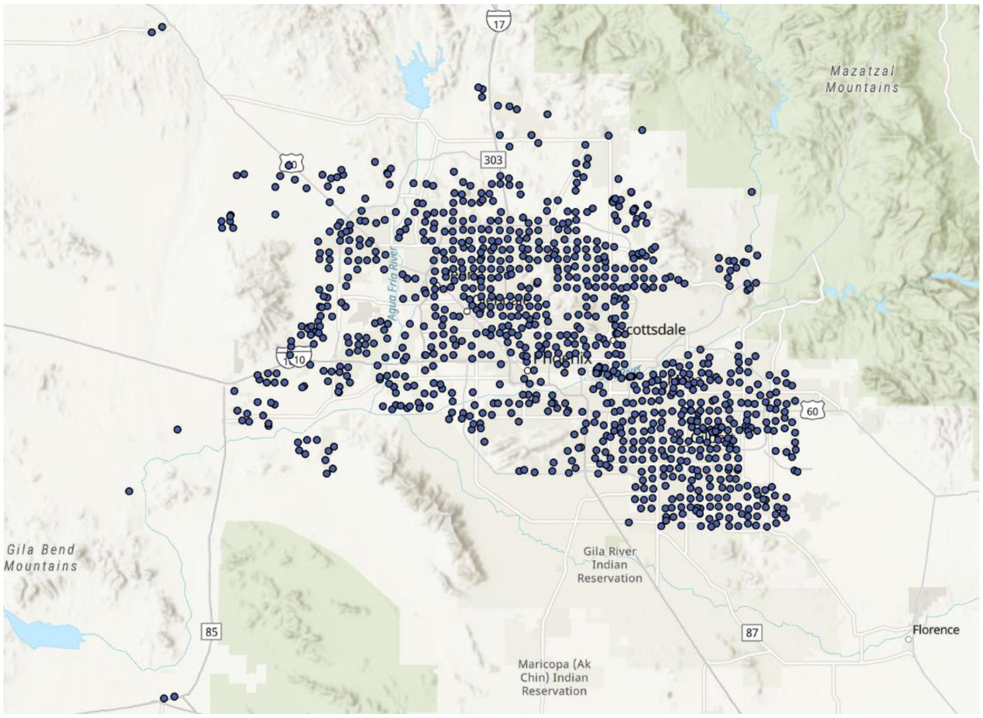

Figure 8: Trapping location points within Maricopa County, Arizona.

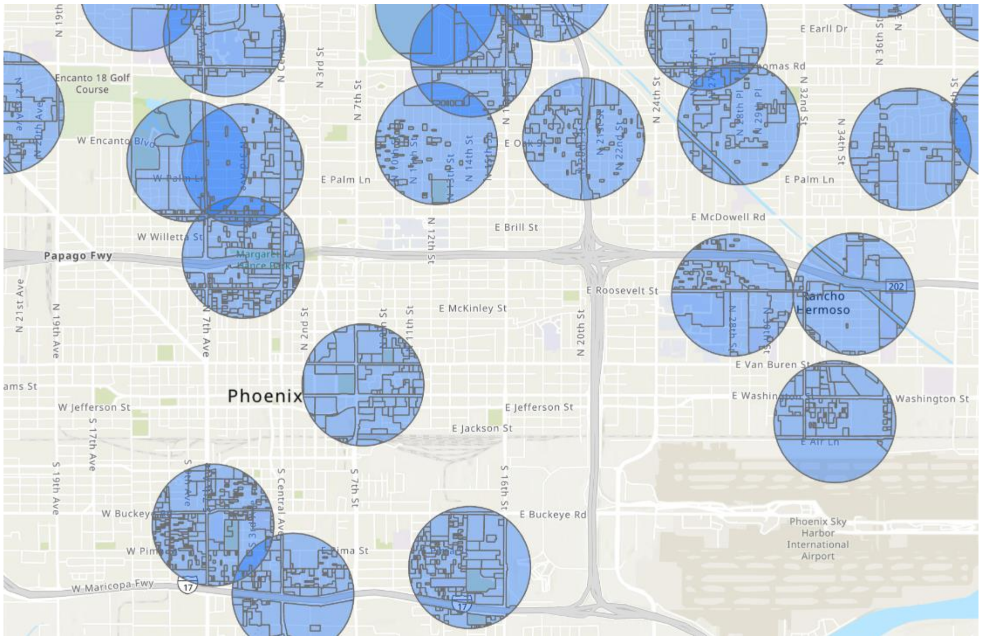

Figure 9: Cx. quinquefasciatus flight range buffers containing land use designations around each trapping location in the greater Phoenix area in Maricopa County, Arizona.

Analysis

For the analysis we looked at two models; a linear regression model for the mean density of females over total trapping events by land use category, and a generalized linear model using binomial logistic regression to determine the odds of finding a positive virus pool when the target species is trapped by land use category. Explanatory variables were log transformed to normalize the data. For the GLM model looking for positive virus, total mean female density was added as an explanatory variable to identify if the number of mosquitoes tested for virus in a pool was a significant factor in finding a pool positive for virus. The data is annual with the following years: 2014, 2016, 2017, 2019, and 2020. Land use category 1 (transportation) was removed from analysis in order to keep the model from achieving a perfect fit due to 100 percent of land use being present for each buffer area. This was the most appropriate category to remove from the study as it included only major roadways. Residential streets that could contain drainage features as a source of larval habitat were classified in land use category 3 and 4 (single and multi-family residential areas). The alpha level was set at 0.05. All data remained included in analysis even if studentized residuals were high. The model was tested for overdispersion and if significant, it was accounted for in the model.

Results

Residential areas, industrial, commercial, and business areas, and vacant or developing areas were highly represented in overall land use (Table 2) with single family residential areas being the most prevalent. The number of sites, mean females trapped, and positive virus pools by year for each species is found in Table 3. Number of trapping sites increased over the years. The highest mean *Cx. quinquefasciatus* numbers were in 2014 and the lowest in 2016. The highest mean *Cx. tarsalis* numbers were in 2019 and the lowest in 2014. The total number of positive virus pools was highest for both species in 2019, and lowest in 2016.

Mean Land Use Percentages for All Trapping Locations for All Study Years

	Land Use Category 1	Land Use Category 2	Land Use Category 3	Land Use Category 4	Land Use Category 5	Land Use Category 6	Land Use Category 7	Land Use Category 8	Land Use Category 9	Land Use Category 10	Land Use Category 11
Mean	4.78	12.77	53.52	4.82	3.99	3.45	2.69	4.03	1.34	7.33	1.29
Median	3.42	8.61	56.24	0.85	0.00	1.64	0.00	0.00	0.00	2.25	0.00
Min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Max	56.70	83.88	97.77	65.05	99.89	52.06	43.16	88.99	34.42	86.60	58.35

Table 6: Land Use Percentage descriptive statistics for all sites for all study years.

		Culex quinquefasciatus		*Culex tarsalis*	
Year	Total Sites Evaluated	Mean Females Trapped	Total Positive Viral Pools	Mean Females Trapped	Total Positive Viral Pools
2014	511	1042.04	139	249.40	37
2016	755	691.70	73	414.42	34
2017	770	887.49	266	919.73	107
2019	803	880.96	416	1031.51	215
2020	801	698.85	156	691.35	54

Table 7: The number of sites, mean females trapped, and positive virus pools by year by species.

The linear regression model found both significant positive and negative associations between land use and *Culex* spp. female density (Table 4 and 5). For both species, mean female mosquito density was positively associated with land use designated as agriculture for all study years. Active open space was also positively associated with *Cx. quinquefasciatus* in three of the five years, but not *Cx. tarsalis*. For both species there was a significant negative association with land use designated for single family residential areas for all study years, and for *Cx. tarsalis* multi-family residential areas for most study years. There was also a negative association with golf courses and vacant space for both species for the majority of study years. Additional associations were significant between the species, but only for select years.

Mean Density of Females for All Trapping by Land use Category										
Land use Category	2014		2016		2017		2019		2020	
	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq
2	0.132	<0.0001					0.0558	0.0069	0.048	0.0131
3	-0.129	0.0006	-0.134	<0.0001	-0.118	<0.0001	-0.146	<0.0001	-0.130	<0.0001
4	-0.081	0.0038								
5	0.097	0.0004	0.056	0.001	0.095	<0.0001	0.068	0.0002	0.093	<0.0001
6					0.090	<0.0001	0.051	0.0143	0.051	0.0062
7			-0.051	0.0049			-0.040	0.0296	-0.037	0.0249
8							-0.035	0.0308	-0.039	0.0095
9										
10			-0.078	<0.0001	-0.077	<0.0001	-0.075	<0.0001	-0.062	0.0002
11							0.109	0.0004		

Table 8: Mean density of Cx. quinquefasciatus females by land use category

Mean Density of Females for All Trapping by Land use Category										
Land use	2014		2016		2017		2019		2020	
	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq
2					-0.058	0.0018				
3	-0.1399	<0.0001	-0.170	<0.0001	-0.246	<0.0001	-0.236	<0.0001	-0.164	<0.0001
4	-0.034	0.0233			-0.042	0.012	-0.063	0.0005	-0.054	0.0002
5	0.098	<0.0001	0.048	0.0003	0.101	<0.0001	0.114	<0.0001	0.056	0.001
6										
7			-0.050	0.0003	-0.073	<0.0001	-0.069	0.0004	-0.042	0.0082
8	0.057	0.001								
9			0.039	0.0172	-0.053	0.0433				
10	-0.055	0.0002	-0.077	<0.0001	-0.122	<0.0001	-0.104	<0.0001	-0.051	0.0008
11	-0.050	0.0175							-0.058	0.0248

Table 9: Mean density of Cx. tarsalis females by land use category

The logistic regression model found both increased and decreased odds for particular land use categories over the study years (Tables 6 and 7). Many locations were only significant for a single year at a time. For *Cx. quinquefasciatus*, increased odds for positive virus pools were found in single family residential areas, agriculture, and active open space land use categories. Decreased odds were associated with industrial, commercial, and business areas, golf courses, inactive open space areas, and vacant areas. For *Cx tarsalis*, increased odds were associated with agriculture, multi-family residential areas, and developing areas. Decreased odds were associated with inactive open space, golf courses, developing areas, and multi-family residential areas. Developing areas and multi-family residential areas had either increased or decreased odds depending on the study year. In addition, for both species, the odds that a mosquito at a site tested positive for a virus increased with the mean number of females caught at that site.

Odds of Finding a Virus Positive Mosquito When Trapped										
Land use	2014		2016		2017		2019		2020	
	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq
2							-0.315183	0.0361		
3					0.647	0.0164				
4										
5					0.301	0.0329				
6									0.699	0.0074
7							-0.278	0.0445		
8							-0.316	0.0213		
9										
10									-0.649	0.0049
11										
Mean Females	1.273	<0.0001	1.627	<0.0001	1.762	<0.0001	1.610	<0.0001	2.007	<0.0001

Table 10: Odds of finding a mosquito pool positive for WNV or SLEV in Cx. quinquefasciatus by land use category. 2014 only looked at WNV, whereas all other years looked at WNV and SLEV.

Odds of Finding a Virus Positive Mosquito When Trapped										
Land use	2014		2016		2017		2019		2020	
	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq	Estimate	Prob>ChiSq
2										
3										
4							-0.705	0.0006	0.776	0.0209
5							0.345	0.0133	0.648	0.0197
6										
7									-441.237	0.0062
8					-0.968	0.002				
9										
10										
11	-3.249	0.0131			0.692	0.0185				
Mean Females			1.272	0.0026	1.785	<0.0001	1.486	<0.0001	1.833	<0.0001

Table 11: Odds of finding a mosquito pool positive for WNV or SLEV in Cx. tarsalis by land use category. 2014 only looked at WNV, whereas all other years looked at WNV and SLEV.

Discussion

This study found a significant association between land use categories and mosquito density. Most importantly we found a strong association with agricultural land use areas for both species and active open space for areas *Cx. quinquefasciatus*. In the desert southwest it is especially important that mosquitoes have shelter for resting out of direct sunlight as the intense sun and heat can exacerbate the desiccation of the insect, especially in low-humidity conditions. These two land use categories would provide a higher presence of shelter and resources over others. The association of higher mosquito densities with agriculture land use is expected and has been documented in other studies throughout the United States (Crowder et al., 2013; Eisen et al., 2010; Gardner et al., 2014) for *Culex* species. The shaded shelter among crop vegetation and equipment along with the watering of crops provide a humid, shaded environment that reduces mortality from desiccation. Irrigation of crops can produce standing water sources that reside long enough for the mosquitoes to complete their immature life stage. The water and crops themselves may attract many types of bird species that prefer to feed on seeds and other agricultural products, especially Passeriformes which are a preferred *Culex* host, promoting host-vector contact. While *Cx. tarsalis* is normally associated with agriculture the utilization of these areas is common for *Cx. quinquefasciatus* (Bhattacharya et al., 2016; Noori et al., 2015) In the Phoenix metropolitan area, the presence of agricultural sections scattered among the urban environment could provide a more suitable habitat for *Cx. quinquefasciatus* compared to the urban areas that they normally stay within (Deichmeister & Telang, 2011; Savage et al., 2014). Similarly, active open space which includes city/regional parks, playgrounds/fields, and local/neighborhood common areas were also positively associated with *Cx. quinquefasciatus* density. Required habitat components may not be present in many of the land use categories that we found to be negatively associated with mean mosquito density. Most surprisingly, there was a negative association with single family residential areas for both species. It is important to note that these areas do not include the neighborhood parks and common areas as those are categorized as active open space. In the Phoenix metropolitan areas, while there are homes that have grass lawns and supportive vegetation, the majority of homes utilize xeriscaping which is a type of landscaping that reduces or eliminates the need for irrigation, instead designing landscapes that are similar to the natural desert environment around them. Many homes can still provide adequate larval habitat for mosquitoes through the use of water holding containers on property that collect precipitation during rainfall (Muller et al., 2010; Santana-Martinez et al., 2017; Walker et al., 2018). Xeriscaping can also deter birds from visiting or roosting on the property if conditions are not right. However, while many homes utilize this type of landscaping, many times there will be public active open space areas that have parks containing irrigated grass and other vegetation.

There were several years in which *Cx. quinquefasciatus* density was positively associated with industrial, commercial, and business areas. There could be large differences in the properties of land use designation that were clustered into this category. Some commercial areas or business parks could provide adequate habitat for mosquitoes and their preferred bird hosts, while others may not. Future research should look at reducing the cluster of this category to determine which areas specifically are driving increased mosquito density.

The lower mosquito densities associated with vacant space are understandable as suitable habitat may not be available. Unkept desert areas do not provide the resources that mosquito populations require.

Interestingly, golf courses were negatively associated with mosquito density. While golf courses provide good mosquito habitat with the presence of vegetation, shelter, and standing water, golf courses typically employ both mosquito control as well as deterrence tactics to keep certain birds from establishing on the property. Many also use pesticides on grasses that can be detrimental to wildlife; especially those that feed on the vegetation (Spurr et al., 2020). Further research should be conducted on vector/host interactions on golf courses within the desert southwest and the prevention measures that they employ.

The logistic regression model showed significant associations between virus presence and land use. The majority of these associations, however, were not consistent between years. This could be due to erratic virus presence within bird populations, or low viremic circulation between years. While the odds of virus presence with land use category was not as consistent as density associations, this study does indicate that higher mosquito density areas do increase the risk of viral transmission. Other studies have found that WNV prevalence has also been found to be associated with specific types of agricultural land use throughout the United States (Bowden et al., 2007; Crowder et al., 2013; Wimberly et al., 2003). In the desert southwest, the factors associated with the incidence of virus presence in vectors needs to be further explored.

Trap location and the number of traps being surveyed differed between years and this variation in data kept us from being able to pool data and run larger multi-year models. At this time, the analysis has not been adjusted for spatial autocorrelation, but preliminary results indicate that spatial autocorrelation in this analysis is minimal and will not impact the results.

The consistent positive associations that we found between mosquito density and agricultural areas and parks/active open space indicate that measures for preventing disease transmission should be focused on these areas. This is especially true during years where mosquito screening reveals high incidence of both virus and vector presence. Having species thresholds in place that trigger intervention strategies can be an effective way to reduce risk as increased densities are associated with increased viral presence.

Chapter 3 - Pyrethroid Resistance in *Culex quinquefasciatus* Populations in Maricopa County, Arizona

Abstract

Arizona has experienced record-breaking numbers of human cases of West Nile virus (WNV) in recent years. Therefore, effective measures to control the responsible *Culex* mosquito populations are essential to reduce disease burden. Maricopa County Vector Control (MCVC) has a comprehensive control program throughout the greater Phoenix area that includes over 800 CO_2 baited mosquito traps that are set on a weekly basis. When a trap either tests positive for a virus, or traps 30 or more mosquitoes in a single trapping, an adulticide spray event with an Ultra Low Volume (ULV) truck mounted fogger is conducted in part or all of the 1 mile square block associated with the surveillance trap. MCVC has almost exclusively utilized pyrethroid class insecticides for the past twenty years. To test the efficacy of the adulticide compounds, we collected immature mosquitoes from six 1-mile square blocks in the city of Chandler, Arizona. All larvae collected were identified as *Culex quinquefasciatus* and were transported to the University of Arizona and were raised to adults. The adult mosquitoes were then tested against the active ingredients of the adulticides being used by MCVC by means of CDC bottle bioassay. We found very high levels of resistance to all active compounds in all mosquito colonies both with and without the use of a synergist. Field trials need to be conducted to gauge the efficacy of the formulated compounds in a working environment. In addition, research needs to be conducted on the populations of mosquitoes within the greater Phoenix area in order to find an effective adulticide so that proper insecticide rotations can occur.

Introduction

West Nile virus (WNV; Flavivirus, Flaviviridae) arrived in the northeast United States in 1999 and quickly spread west, reaching Arizona in 2003. Between the years of 2003 and 2020, Arizona had an overall yearly average of 101 human cases and 7 deaths. The 2021 season produced a record-breaking 1693 cases and 101 deaths, which increased the average to 190 human cases and 14 deaths, nearly doubling the average of the previous eighteen years in one season (Arizona Department of Health Services, 2023). *Cx. quinquefasciatus* and *Cx. tarsalis* are the most common *Culex* species found throughout the state of Arizona and are the species associated with WNV transmission. *Cx. quinquefasciatus* have routinely been known to be associated with urban environments (Deichmeister & Telang, 2011; Savage et al., 2014), while *Cx. tarsalis* have been known to prefer rural areas (Chuang et al., 2011; Griffin D., 2008; Larson et al., 2010; Schurich et al., 2014), however some overlap can occur.

Integrated vector management

In order to combat the spread of WNV, many counties in Arizona conduct routine surveillance in order to identify areas in which the virus may be circulating. This surveillance can include mosquito trapping and testing, dead bird testing, or sentinel chicken testing since birds are the preferred host of most *Culex* mosquitoes. If a test comes back positive for WNV, preventative or control measures can be conducted based on the resources available to the county. Most smaller counties in Arizona that conduct surveillance rely on public education campaigns in areas where a positive sample was identified. This can include education in how to remove larval habitat, and how to reduce mosquito bites with clothing coverage, increased housing barriers, and repellant use. For counties with more comprehensive programs, the presence of virus in a mosquito pool, or identification of an area with a high mosquito density can lead to mosquitoes being targeted and killed through ultra-low volume (ULV) insecticide fogging by either truck mounted, or aircraft mounted devices.

ULV insecticides work when the spray droplets come into contact with the adult mosquito, so it needs to be applied when the mosquito is most active and is most likely to be exposed. This means that spray events need to be tailored to the species being targeted (Bengoa et al., 2014; Chaskopoulou et al., 2011; Groves et al., 1997; Mani et al., 2005; Mount et al., 2006; Trout et al., 2007; Yap et al., 1997). *Culex* mosquitoes prefer to host seek after dusk with peak activity one to three hours after sunset during the warmest, driest part of the night (Reisen et al., 1997) when birds are likely roosting. In order to successfully target *Culex* species, nighttime spraying is required. However, adulticide spraying is transient and without constant reapplication, mosquito populations will return to an area after a few days as immatures within that area develop and eclose as adults. Due to this, most jurisdictions reserve the use of ULV spraying to situations of increased human risk such as a positive mosquito pool or a high density of mosquitoes within an area.

Insecticide resistance in Culex species

Pyrethroids and organophosphates (OPs) are the most common insecticides used in the United States, with pyrethroid use being more frequent due to lower risk to humans. Pyrethroids are synthetic formulations based on naturally occurring insecticides in plants. They are safe to use around mammals but are toxic to insects and aquatic life (Jolly et al., 1978). When an insect is exposed to a pyrethroid, the voltage gated sodium channel (NaV) in the neural membranes is targeted (Martinez-Torres et al., 1999; Scott et al., 2015), and the open state of the channel is prolonged. This results in a repetitive firing of the nervous impulse, causing involuntary muscle spasms, exhaustion and eventually death. This event is known as the knockdown effect (kd) (Bloomquist, 1996). When mosquitoes become resistant to pyrethroids, they become known as knockdown resistant (kdr). Mutations pertaining to pyrethroid resistance usually occur in the IIS6 region in *Culex* mosquitoes (Brito et al., 2013; Chen et al., 2010; Hardstone et al., 2007; Yoshimizu et al., 2020) with a shift in nucleotide bases from A to T that converts wild-type amino acid Leucine (L) to phenylalanine (F) at codon 1014 (Scott et al., 2015).

OPs are less frequently used synthetic chemicals that are toxic to a wide range of animals and plants. Upon entering the body, the insecticide inhibits acetylcholinesterase activity, which creates an accumulation of acetylcholine, a neurotransmitter, in the synapse and neuromuscular junctions (Chambers et al., 2010). This buildup causes the continuous firing of nerve impulses which overload the nervous functions in the body causing death of the insect. OPs are also associated with adverse health effects in humans and other animals (CDC, 2022b).

OP resistance is attributed to the overproduction in α-esterase and β-esterase activity that bind to the OP that is inhibiting the acetylcholinesterase activity. Once bound, the esterase can remove the OP, reactivating the acetylcholinesterase's ability to cleave acetylcholine (Bisset et al., 1990; Mouches et al., 1987; Raymond et al., 1987).

Formulated insecticide compounds often include synergists or inhibitors that enhance the efficacy of the active ingredient. These compounds inhibit enzymes which are involved in the detoxification and metabolism of insecticides (Hemingway & Ranson, 2000). Piperonyl Butoxide (PBO) inhibits oxidase enzymes that detoxify pyrethroids.

S.S.Stributlyphosphorotrithioate (DEF) inhibits esterase enzymes that detoxify OPs (Richards et al., 2020).

Measuring Resistance

The CDC bottle bioassay is a standard method of determining mosquito susceptibility or resistance to specific insecticides (CDC, 2021a). It is a laboratory-based assay where the chemical(s) to be analyzed coat the inside of a glass bottle and the mortality of mosquitoes placed in the bottle is recorded over a two-hour period. Mortality times are compared to a CDC diagnostic table that provides the expected 100% mortality times for susceptible mosquito species when exposed to the recommended diagnostic dose concentrations of specific insecticides. Additional bottles using acetone, which is non-lethal to mosquitoes, is used as a negative chemical control, and a known susceptible, or non-resistant laboratory strain of each species is used as a positive control. Calibration of the assay may be required due to variations in the susceptible strain being used. According to CDC, four replicates of each test bottle are ideal to account for uncertainty, however if the number of mosquitoes used for the assay is not adequate for four bottles, fewer bottles can still acceptably be used (CDC, 2021a).

In addition to testing for resistance to particular insecticides, CDC bottle bioassays can also test mosquitoes for the ability to detoxify insecticides by including inhibitors in the test. To test if detoxication is occurring, half of a colony is tested against the insecticide to obtain a baseline resistance profile. The other half of the colony is exposed to the inhibitor in a bottle for one hour, then transferred back to a holding container for an additional hour. Those mosquitoes are then tested against the same insecticides as the first half of the colony and the resistance curves between the two are compared. If resistance is lower in the colony that was exposed to the inhibitor, a detoxification mechanism is involved (CDC, 2021a).

Resistant populations tend to be localized to where the insecticide is being applied due to the short active range of the mosquitoes being targeted. Some mosquitoes have a greater range than others so the spread of resistance can vary depending on species (Brogdon et al., 1988; Knipling, 1952). Due to this, resistance testing in mosquitoes collected from multiple treatment areas is needed.

The objective of this study was to assess the susceptibility of *Cx. quinquefasciatus* to the pyrethroids being applied by Maricopa County Vector Control (MCVC) as they have routinely and consistently used the pyrethroid adulticide compounds Duet and/or Permanone to treat areas for *Culex* mosquitoes for the past decade. *Cx. quinquefasciatus* were the concentration of the study as they may pose a greater risk to human health due to their preference for urban habitats.

Methods

Study Site

The mosquitoes tested were collected in the city of Chandler, part of the greater Phoenix Metropolitan area, in Maricopa County, Arizona. Chandler is located southeast of the Phoenix city center (Figure 1) and had a population of 275,987 in 2020 (US Census Bureau, 2022). The climate in Maricopa County is a sub-tropical desert type with low annual rainfall (6.82 inches average over the past 10 years) and low relative humidity (44% average). Daytime temperatures average 93.4°F throughout the summer months over the past 10 years with monsoon rainfall occurring from July through October.

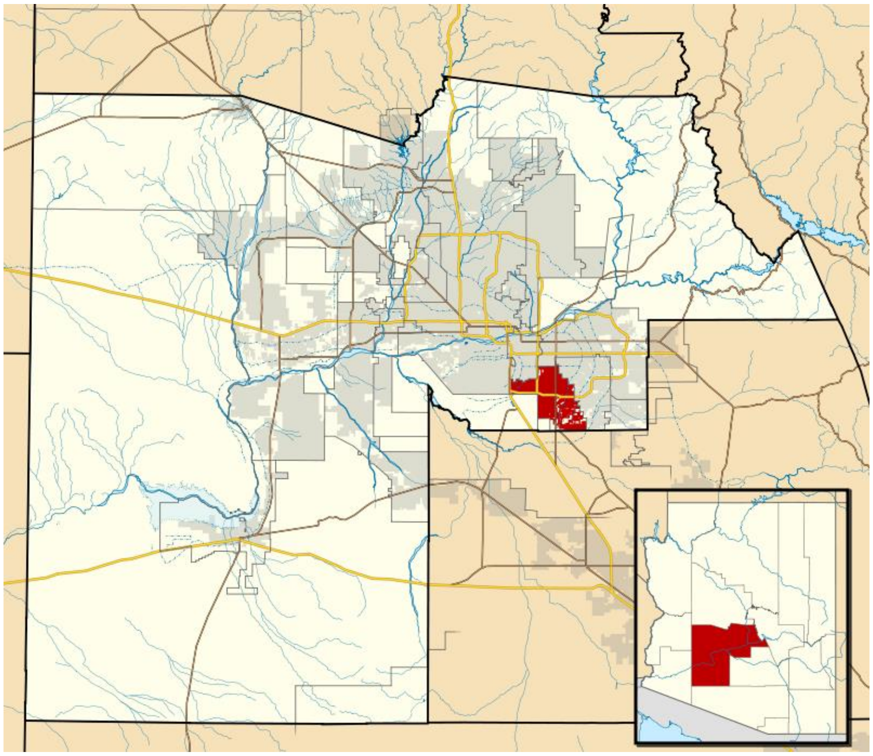

Figure 10: The City of Chandler within Maricopa County, Arizona.

MCVC has an extensive mosquito control program that currently includes over 800 CO2-baited surveillance traps throughout the greater Phoenix area. Each surveillance trap is situated within a one-mile-square block and is checked weekly throughout the year. MCVC uses the traps to monitor mosquito and arbovirus activity and maintains specific thresholds that trigger adult mosquito spraying events. Specifically, if a pool of *Culex* mosquitoes collected from a single trap test positive for either West Nile virus or St. Louis encephalitis, an adulticide spray event using a truck mounted ULV fogging device within that mile block is scheduled. Furthermore, if the number of a specific species per trap exceeds a predetermined threshold, a spray is scheduled. A spray event typically occurs 24 – 72 hours after the event is scheduled due to time needed for staff allocation and resident notification. Events can be accelerated and completed within 24 hours in emergency situations and may be delayed up to a week depending on weather conditions or other factors.

While the overuse of pyrethroids can lead to resistance, MCVC state that they have completed field trails where caged wild mosquitoes are placed within the location that they were captured and are exposed to a fogging event. Mortality is then assessed 24 hours after the event. MCVC has stated that the pyrethroid adulticides being used are still effective after assessment of these field trials. However, routine insecticide resistance testing is not conducted.

Mosquito collection and rearing

Larvae were collected from drainage features in six different one-mile-square blocks in Chandler, Arizona (Figure 2) and transported to the University of Arizona insectary. A sample of the larvae were identified in each collection to verify species. Larvae were fed ground cat food and kept in a rearing chamber at 19°C-30°C, 31%-99% relative humidity, and a 16:8 (Light: Dark) hour cycle. Larval containers were checked for pupae regularly and when present, they were harvested and moved to emergence cages. Adult mosquitoes were fed a 10% sucrose solution daily. Eggs from the Johannesburg (JHB) susceptible strain NR-43025 of *Cx. quinquefasciatus* were obtained from BEI Resources, NIAID, NIH and were hatched, reared, and bred according to CDC provided instructions in the insectary under the same conditions until an adequate number of adults were obtained for bioassays. Study mosquitoes were roughly 50 days old when tested due to the delay in propagating the susceptible colony.

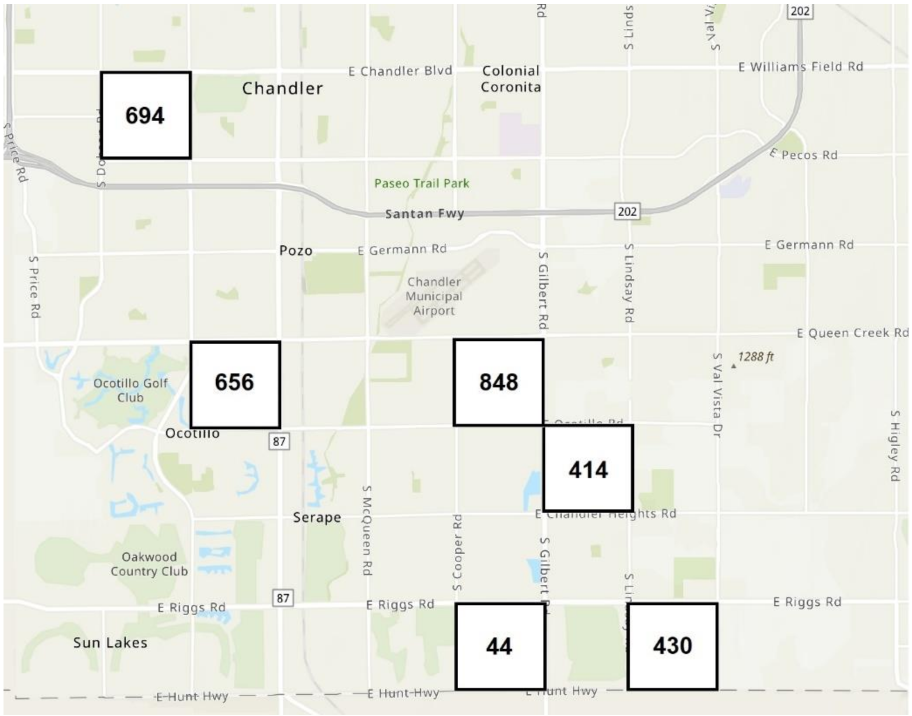

Figure 11: Larval collection blocks within the City of Chandler, Arizona.

Bottle bioassays

Following the CDC bottle bioassay procedure and working in a fume hood, 250ml glass Wheaton bottles were coated with 1ml of pesticide solution composed of the diagnostic dose of the chemical being tested (Table1) and acetone. The active ingredients in the Duet compound are prallethrin and d-phenothrin (Sumethrin), with piperonyl butoxide (PBO) being included as an inhibitor. Permanone's active ingredient is permethrin with PBO as an inhibitor. Each active ingredient was tested in the bioassay independently at their diagnostic dose. The diagnostic dose for prallethrin is 0.05µg/ml per bottle, d-phenothrin is 20µg/ml per bottle, permethrin is 43µg/ml per bottle, and PBO is 400µg/ml per bottle. Bottles were covered from light exposure until dry. Only three of the six test colonies were exposed to the inhibitor PBO due to limited mosquito numbers. To test the efficacy of the inhibitor, half of a colony was placed in a 500ml Wheaton flask that was coated with the diagnostic dose of PBO (Table 3.1) and left for one hour. The colony was then moved back to a holding container for an additional hour. Three replicates for each site (six from sites tested with the inhibitor; three with exposure, three without) containing an average of 11 mosquitoes ranging from 7 to 17 mosquitoes were aspirated into each bottle and tested against each active ingredient. Mortality was assessed every 15 minutes up to 120 minutes. According to CDC, mosquitoes are considered dead if they express erratic flight or can no longer stand. These are indicators that the insecticide is effectively impacting the nervous system of the insect which will lead to mortality. A control bottle containing only acetone for each assay was included.

The JHB susceptible colony was evaluated at the diagnostic concentration for each active ingredient to determine if 100% mortality was achieved at the provided CDC diagnostic time. JHB mortality was similar to the CDC published table, so calibration was not required.

Mean percent mortality for each active ingredient, with and without pre-exposure to the inhibitor, at the diagnostic dose was recorded for each time interval after exposure. The World Health Organization (WHO) defines 97% - 100% mortality at the recommended diagnostic time as being susceptible. 90% - 96% mortality at the recommended diagnostic time indicates the population is developing resistance. Less than 90% morality at the recommended diagnostic time implies resistance (CDC, 2022a).

Chemical diagnostic dose and susceptible *Cx. quinquefasciatus* 100% mortality times

Chemical	Final Concentration/Bottle µg/bottle	*Cx. quinquefasciatus* Susceptible Colony 100% Expected Mortality Time (Minutes)
Sumithrin	20	45
Prallethrin	0.05	60
Permethrin	43	30
Piperonyl butoxide (PBO)	400	-[1]

Piperonyl butoxide (PBO) is used as an inhibitor for resistance mechanisms only.

Table 12: Chemical diagnostic dose and susceptible Cx. quinquefasciatus 100% mortality times

Statistical Analyses

A Kaplan-Meier survival analysis with log-rank and Wilcoxon tests were used to determine whether the field test populations exhibited a significant difference in mortality at both the chemical specific diagnostic time and at the conclusion of the test (120 minutes) compared to the known susceptible JHB colony for each chemical routinely used by MCVC. In addition, differences between colonies at both time increments, and the difference in mortality between colonies that were exposed to the PBO and those that were not, were assessed.

Results

Percent mortality at the diagnostic time and at the end of the test (120 minutes) is displayed in Table. 2 for the susceptible colony and for each of the test collection sites. Mortality curves without and with the use of the inhibitor for the three pyrethroid insecticides are shown in Figs. 3-8. All assays, despite the use of an inhibitor, indicate a high level of resistance in all sites tested. In the absence of the inhibitor, we found an average mortality of 0.5% for Prallethrin, 1.5% for Permethrin, and 1.67% for Sumithrin at the diagnostic time across the different field test populations. In the presence of the inhibitor, we found an average of 15.33% for Prallethrin, 16.67% for Permethrin, and 24.67% for Sumithrin at diagnostic time across the collections. While Sumithrin with PBO provided the highest percent mortality at the diagnostic time, mortality failed to reach 100% at the end of the test period. While Permethrin had a lower percent mortality at the diagnostic time it resulted in a higher percentage of mortality at the end of the test cycle. All negative controls had 0% mortality at the end of the test cycle except for one flask in which one mosquito died giving a total mortality of 0.07%.

Percent mortality of *Culex quinquefasciatus* mosquitoes at diagnostic times and at end of CDC bioassay

	Percent Mortality			
	Without Inhibitor[1]		With Inhibitor[1]	
	Diagnostic Time[2]	120 Minutes	Diagnostic Time[2]	120 Minutes
Permethrin (20 µg/ml)				
JHB Colony[3]	100	100	100	100
Maricopa Site 044	0	19	-	-
Maricopa Site 414	0	14	18	100
Maricopa Site 430	0	11	18	100
Maricopa Site 656	7	10	14	86
Maricopa Site 694	0	34	-	-
Maricopa Site 848	2	12	-	-
Prallethrin (0.05 µg/ml)				
JHB Colony[3]	100	100	100	100
Maricopa Site 044	3	6	-	-
Maricopa Site 414	0	0	21	68
Maricopa Site 430	0	0	11	48
Maricopa Site 656	0	0	14	36
Maricopa Site 694	0	0	-	-
Maricopa Site 848	0	3	-	-
Sumithrin (43 µg/ml)				
JHB Colony[3]	100	100	100	100
Maricopa Site 044	4	4	-	-
Maricopa Site 414	0	10	28	86
Maricopa Site 430	0	19	14	90
Maricopa Site 656	3	10	32	88
Maricopa Site 694	0	4	-	-
Maricopa Site 848	3	8	-	-

1. Piperonyl butoxide (PBO) is used as an inhibitor.
2. CDC diagnostic time is 30 minutes for permethrin, 45 minutes for sumithrin, and 60 minutes for prallethrin.
3. JHB susceptible colony from BEI Resources

Table 13:Percent mortality of Culex quinquefasciatus mosquitoes at diagnostic times and at end of CDC bioassay

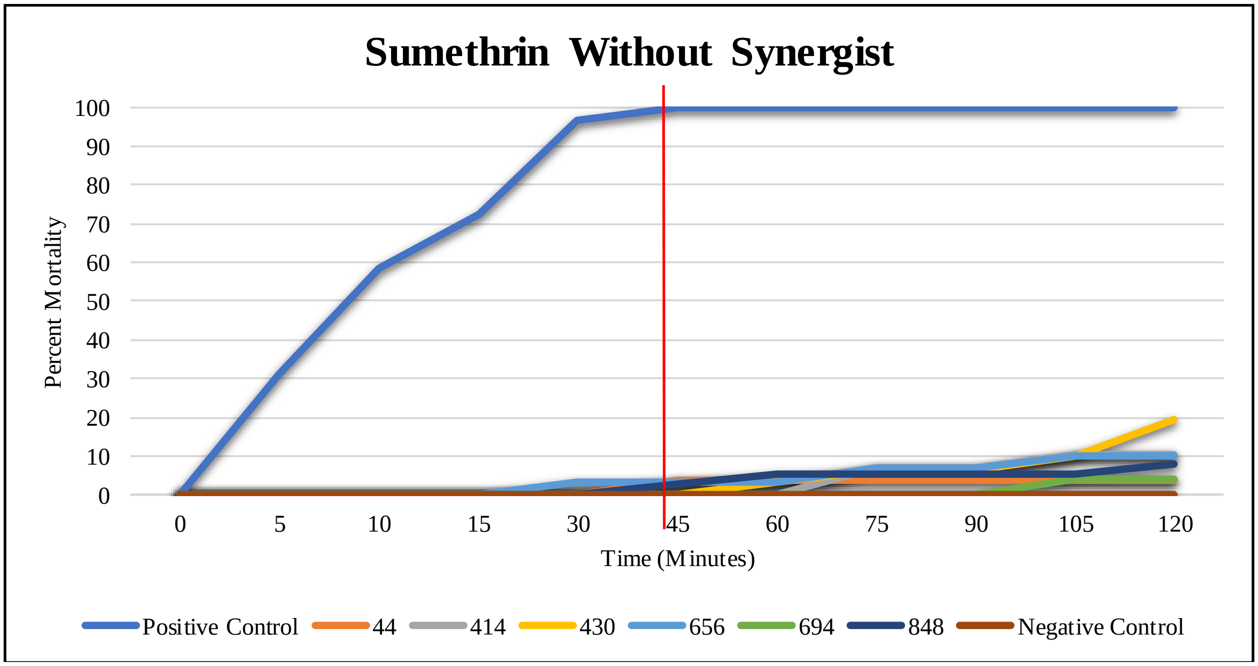

Figure 12: Morality curve for the diagnostic dose of Sumethrin without synergist. Black line represents diagnostic time for 100% mortality in a susceptible colony.

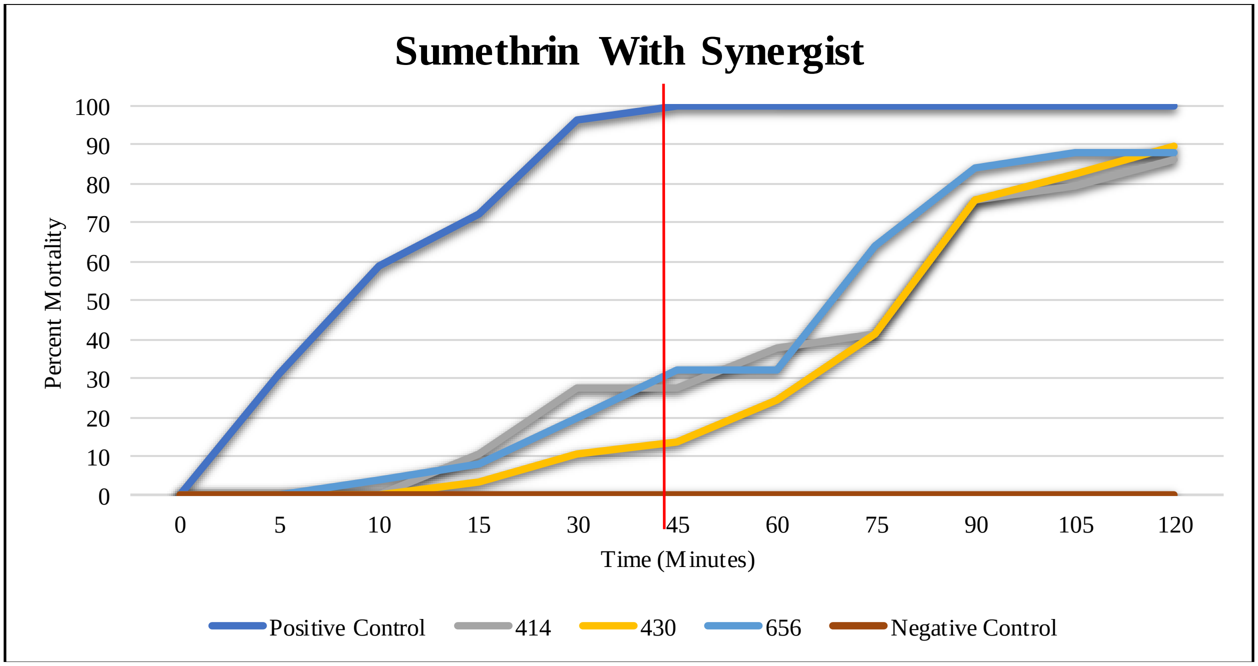

Figure 13: Morality curve for the diagnostic dose of Sumethrin with synergist. Black line represents diagnostic time for 100% mortality in a susceptible colony.

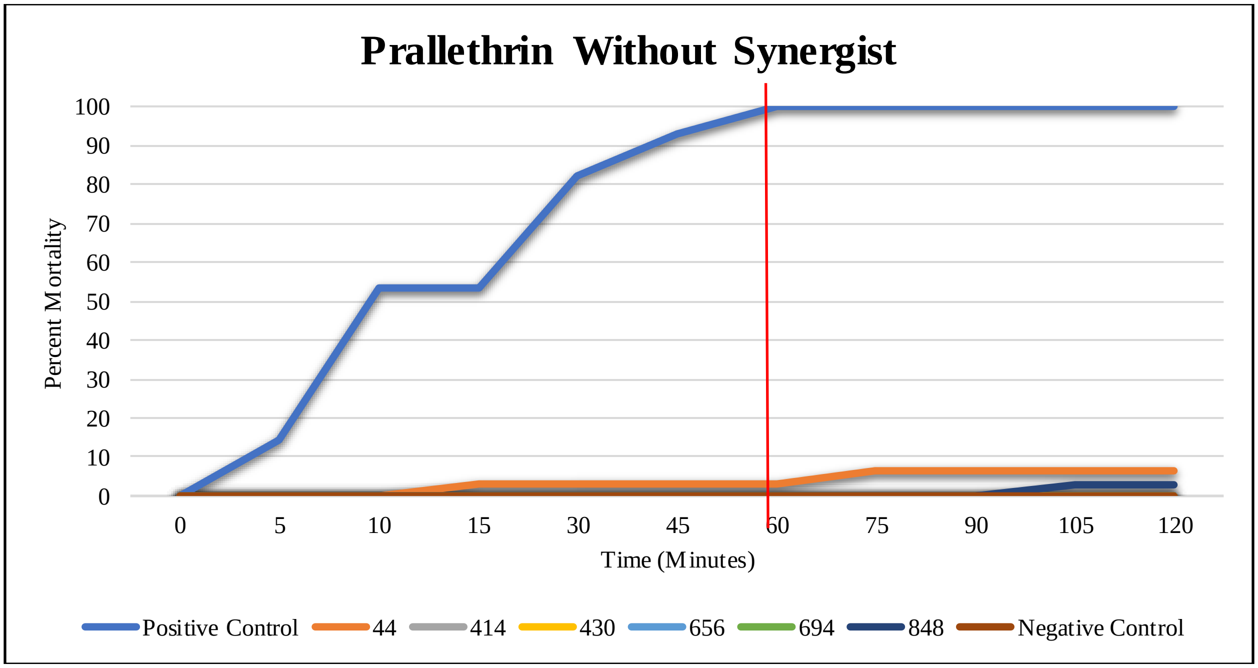

Figure 14: Morality curve for the diagnostic dose of Prallethrin without synergist. Black line represents diagnostic time for 100% mortality in a susceptible colony.

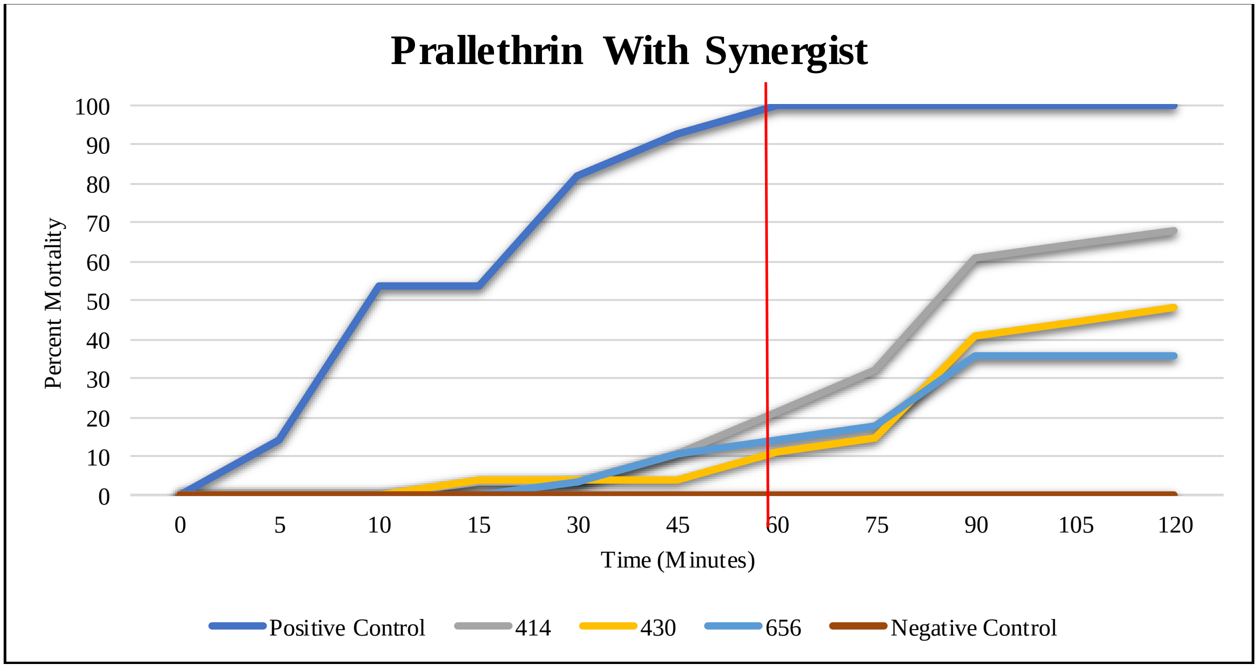

Figure 15: Morality curve for the diagnostic dose of Prallethrin with synergist. Black line represents diagnostic time for 100% mortality in a susceptible colony.

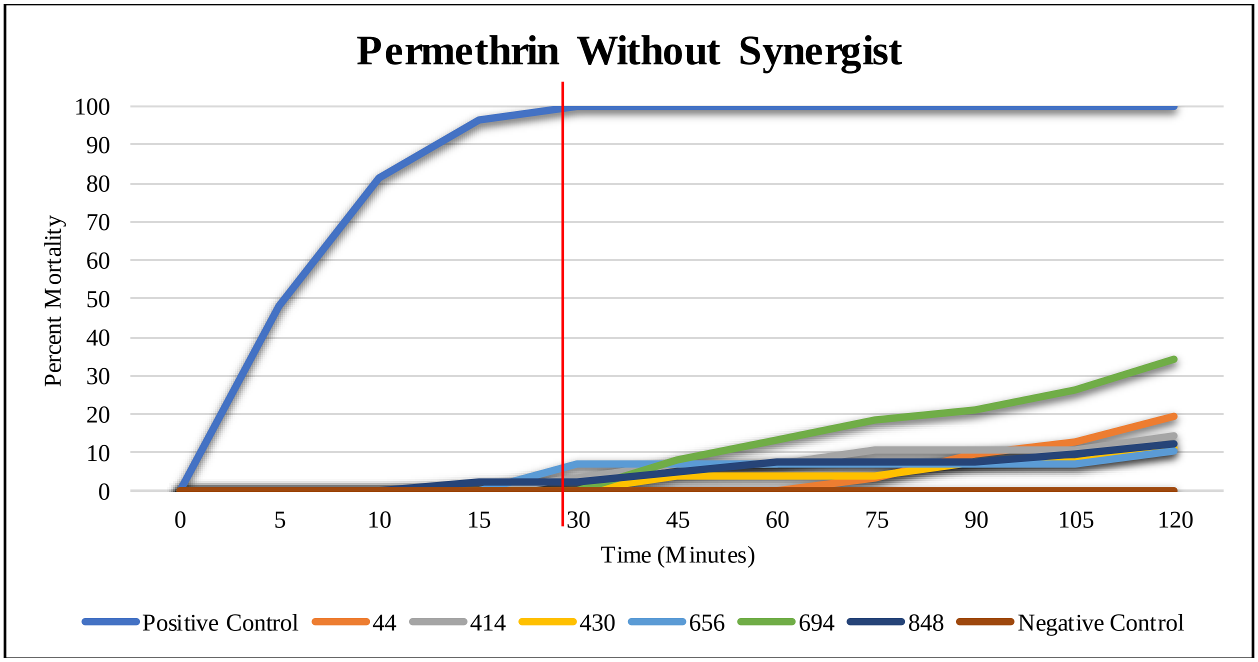

Figure 16: Morality curve for the diagnostic dose of Permethrin without synergist. Black line represents diagnostic time for 100% mortality in a susceptible colony.

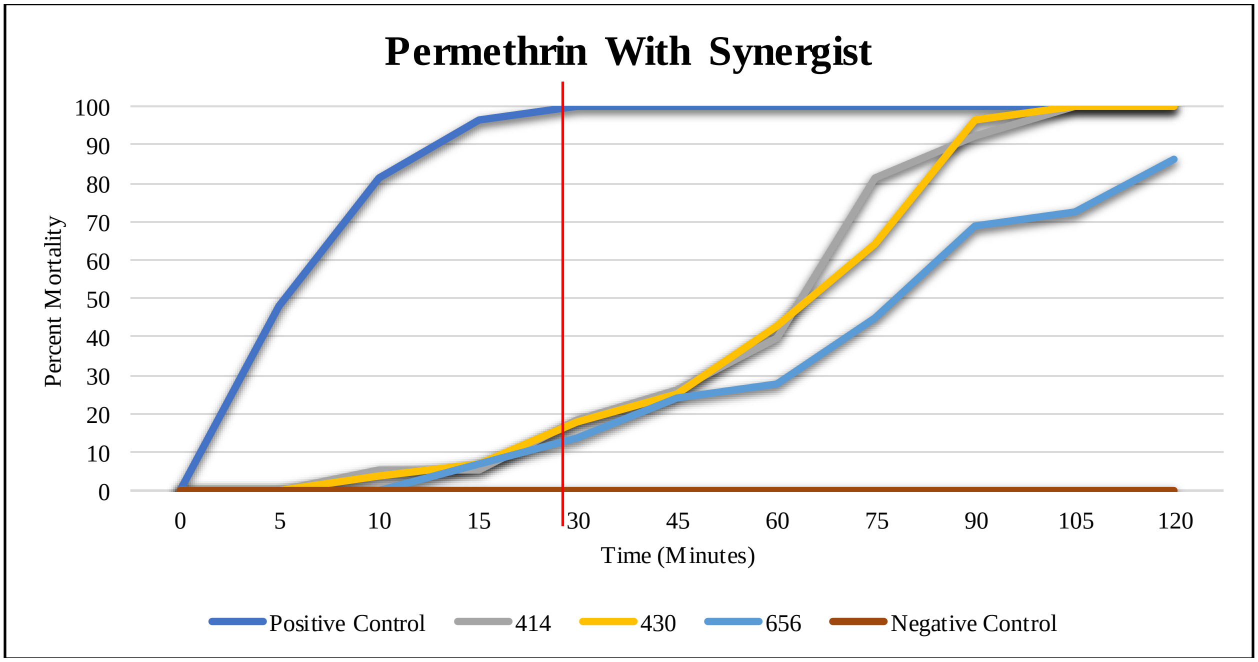

Figure 17: Morality curve for the diagnostic dose of Permethrin with synergist. Black line represents diagnostic time for 100% mortality in a susceptible colony.

Survival analysis found that in the absence of the inhibitor there were no significant differences in survival between the test colonies at either the diagnostic time, or at the end of the test at 120 minutes (Table 3) with the exception of permethrin at 120 minutes of exposure to just the active ingredient concentrations. There was no significant difference between test colonies exposed to the inhibitor at either the diagnostic time, or at the end of the test at 120 minutes. All test colonies were significantly different from the susceptible colony for both with and without exposure to the inhibitor. All test colonies that were exposed to the inhibitor were significantly different than the colonies that were not exposed with the exception of prallethrin at the diagnostic time.

Kaplan-Meier Survivability Analysis

Survivability Test	Chemical	Time (Minutes)[1]	DF	Log-Rank		Wilcoxon	
				Chi-Square	P-value	Chi-Square	P-value
Difference between test colonies and control colony	Sumithrin	45	6	303.41	**<0.0001**	291.76	**<0.0001**
Difference between test colonies and control colony	Sumithrin	120	6	303.68	**<0.0001**	291.97	**<0.0001**
Difference between test colonies	Sumithrin	45	5	2.84	0.725	2.84	0.725
Difference between test colonies	Sumithrin	120	5	1.8	0.876	1.77	0.88
Difference between test colonies and control colony	Prallethrin	60	6	299.77	**<0.0001**	287.46	**<0.0001**
Difference between test colonies and control colony	Prallethrin	120	6	299.92	**<0.0001**	287.59	**<0.0001**
Difference between test colonies[2]	Prallethrin	60	5	0	.	0	.
Difference between test colonies	Prallethrin	120	5	4.03	0.545	4.03	0.545
Difference between test colonies and control colony	Permethrin	30	6	310.04	**<0.0001**	297.96	**<0.0001**
Difference between test colonies and control colony	Permethrin	120	6	318.55	**<0.0001**	305.21	**<0.0001**
Difference between test colonies	Permethrin	30	5	3.73	0.559	3.73	0.559
Difference between test colonies	Permethrin	120	5	18.34	**0.0025**	18.19	**0.0027**
Difference between test colonies with inhibitor use and control colony	Sumithrin	45	3	112.16	**<0.0001**	106.25	**<0.0001**
Difference between test colonies with inhibitor use and control colony	Sumithrin	120	3	111.65	**<0.0001**	105.85	**<0.0001**
Difference between test colonies with inhibitor use	Sumithrin	45	2	2.09	0.352	2.12	0.347
Difference between test colonies with inhibitor use	Sumithrin	120	2	0.42	0.808	0.66	0.719
Difference between test colonies with inhibitor use and control colony	Prallethrin	60	3	128.9	**<0.0001**	121.13	**<0.0001**
Difference between test colonies with inhibitor use and control colony	Prallethrin	120	3	129.78	**<0.0001**	121.81	**<0.0001**
Difference between test colonies with inhibitor use	Prallethrin	60	2	0.61	0.735	0.59	0.743
Difference between test colonies with inhibitor use	Prallethrin	120	2	1.91	0.385	1.98	0.372
Difference between test colonies with inhibitor use and control colony	Permethrin	30	3	143.81	**<0.0001**	135.74	**<0.0001**
Difference between test colonies with inhibitor use and control colony	Permethrin	120	3	146.58	**<0.0001**	138.24	**<0.0001**
Difference between test colonies with inhibitor use	Permethrin	30	2	1.83	0.4	1.85	0.397
Difference between test colonies with inhibitor use	Permethrin	120	2	4.6	0.1	5.62	0.06
Difference in colonies with and without the use of the Inhibitor	Sumithrin	45	5	15.42	**0.009**	15.46	**0.009**
Difference in colonies with and without the use of the Inhibitor	Sumithrin	120	5	45.25	**<0.0001**	44.35	**<0.0001**
Difference in colonies with and without the use of the Inhibitor	Prallethrin	60	5	10.57	0.061	10.54	0.061
Difference in colonies with and without the use of the Inhibitor	Prallethrin	120	5	41.2	**<0.0001**	40.95	**<0.0001**
Difference in colonies with and without the use of the Inhibitor	Permethrin	30	5	12.73	**0.026**	12.74	**0.026**
Difference in colonies with and without the use of the Inhibitor	Permethrin	120	5	85.77	**<0.0001**	82.52	**<0.0001**

1. Analysis was conducted at the diagnostic time for each chemical and at the end of the experiment.
2. All Colonies survived.

Table 14. Kaplan-Meier Survivability Analysis

Discussion

These bioassays have found that *Cx. quinquefasciatus* mosquitoes in Chandler, Arizona exhibited high levels of resistance to the active ingredients in both of the insecticide formulations products that MCVC routinely use for control. The WHO classifies resistance as anything with a mortality of under 90% at the diagnostic time and dose and our study found high levels of resistance despite the use of a synergist for all test sites. Average mortality for all sites assessed was low and only mortality to permethrin at 120 minutes was found to be significantly different between the sites. This implies that most test sites are very similar in their resistance profiles despite how far apart they are from one another. The significant difference in mortality between blocks for permethrin at 120 minutes may be due to the number of times each block had been exposed to Permanone, putting greater stress on the populations to adapt. In the absence of the inhibitor, no mosquito pools from any collection sites achieved 100% mortality at the diagnostic time or at the end of the test. Two collections did exhibit 100% mortality at the end of the test in the inhibited permethrin bioassays. The results indicate greater susceptibility to the Permanone formulation, which may explain why MCVC does not yet see operational failure with Permanone in spite of evolving resistance.

The rotation of insecticides is an important component in vector management. Rotating within the same class of insecticides does not effectively manage resistance or extend the efficacy of the that insecticide class against the target pests. Unfortunately, vector control agencies are often limited in terms of control products that are acceptable for residential space spraying. In Arizona, current adulticides for residential use are limited to specific pyrethroids and only a couple of OPs. Vector control agencies that do not practice insecticide rotations, or practice rotations over an inappropriate time frame can inadvertently select for resistance. The continued use of pyrethroids in this environment will only strengthen the resistance capabilities of the targeted mosquitoes thereby further reducing the efficacy of the spray events. While field trials in some areas are stated to still provide mortality after extended periods of time, the efficacy of the treatment at the diagnostic dose and time reflects that complete mortality may not occur in all areas.

Field efficacy studies are urgently needed to evaluate the usefulness of the compounds that we have found high levels of resistance to. Further studies and bioassays should be conducted to determine which alternative insecticides will be most effective on mosquito populations within Maricopa County before implementation. Once an appropriate chemical is identified, local mosquito populations should be tested for resistance in order to establish a baseline before using the chemical. Routine bioassay testing should then be conducted to monitor for a developing resistance profile within the local populations. We acknowledge that the use of OPs comes with stricter use authorizations and increased health risks. We hope that this data can be used to convey the immediate need of insecticide rotation in a time when *Culex*-borne disease numbers are increasing beyond what has been previously recorded.

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