DISTRIBUTION OF CONTAINER-BREEDING MOSQUITOES IN URBAN AREAS OF SOUTHERN OKLAHOMA

By

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DISTRIBUTION OF CONTAINER-BREEDING MOSQUITOES IN URBAN AREAS OF SOUTHERN OKLAHOMA

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Abstract:
Aedes aegypti, the yellow fever mosquito, is a significant arbovirus vector worldwide and one that has gained prominence recently in the US as a primary vector for Zika virus. In 2016, *A. aegypti* was discovered again in four cities in southern Oklahoma during surveillance activities along with other important container-breeding species, namely *Aedes albopictus* and *Culex pipiens* complex. While pockets of *A. aegypti* in several Oklahoma cities were identified, there is limited understanding of the nature and extent of these populations within given urban areas or regions of the state. In this study, we hypothesized that *A. aegypti* were more likely to occur in the southern part of the state and were more likely to become established within regional urban areas. Between May to August 2017, mosquitoes were collected in six urban areas along two transects in central and western Oklahoma between the Red River (Texas border) and cities 60 miles from the border. Bi-weekly mosquito collection (total 2,118 trap nights) utilized Gravid Aedes traps (GAT) and BG-sentinel traps across urban gradients. With the use of geographical information systems (GIS), predictions of mosquito density in relation to vegetation, container availability and other anthropogenic factors were determined within urban habitats. Of the 6,628 female mosquitoes collected, 80% were container-breeding species (*A. albopictus* and *A. aegypti*) with proportions differing between different urban areas. *Aedes aegypti* was more localized in southern Oklahoma while other container species were more widely distributed. While the prevalence of *D. immitis* in *A. albopictus* and *C. pipiens* complex was low, regression models confirmed significant predictive parameters for container-breeding mosquito species. The results of this study will assist in the prediction of mosquito vector habitat in urban areas of Oklahoma and potentially demonstrate how arboviruses could affect these cities in the event of an outbreak.

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CHAPTER I

INTRODUCTION

Mosquito-borne arboviruses have been a problem throughout the world for millennia, causing humans to develop complex systems to control mosquitoes and limit the extent to which these arboviruses impact our development as a species. This is no less an issue now than in the past in the United States. Hampered by malaria and yellow fever in the initial 200 years of the nation, millions of dollars were spent in the early 1900s to eliminate the breeding sites for the mosquitoes that transmit these diseases, which led to the eradication of these diseases throughout the country. Although small outbreaks of arboviruses occurred, the success of these eradication programs was short-lived with the epidemic of West Nile that swept across the US, starting in New York in 1999 and ending in California in 2003 (CDC, 2018b). West Nile virus continues to be endemic throughout the country. Recent outbreaks of chikungunya and Zika virus in the southern Americas region with the continued threat of Dengue coming into the country via persons travelling to regions experiencing outbreaks or infectious people moving into the US continues to emphasize the need to be vigilant. This increased need for vigilance correlates with an accompanying need to identify where specific competent vectors, specifically, *Aedes* container-breeders, are thriving in local landscapes. The main container breeders in the United States that impact the spread of disease are *Aedes aegypti*, *Aedes albopictus* and *Culex pipiens* complex L.

Aedes albopictus and *C. pipiens* develop quickly in the right conditions and are widely distributed across the southern United States while *A. aegypti* is established in more localized areas such as Florida, Arizona, Texas, and California (Hahn et al., 2017). While these populations are well characterized in other areas, their ecology is not well understood in the Great Plains region. In Oklahoma, *A. albopictus* has been identified in all 11 eco-zones, which demonstrates the ecological flexibility of this container-breeding species (Noden et al., 2015a). *Culex pipiens* has also been reported across the state, but is more localized in the east and central part of Oklahoma (Noden et al., 2015b; Bradt, 2017). In 2016, this scenario was enhanced when *A. aegypti* adults were collected and identified in southern Oklahoma for the first time since the 1940's (Bradt, 2017).

The discovery of *A. aegypti* in Oklahoma lead to many questions as the establishment of the species in Oklahoma may have occurred by multiple introductions from neighboring states or wind-blown populations migrating from Texas. At the time of discovery, Texas confirmed that *A. aegypti* populations were present in most of the counties along the Red River or the Texas border, which correlated with Oklahoma counties where *A. aegypti* were found (Hahn et al., 2017). *Aedes aegypti* was also discovered along the western state border of the Texas panhandle, surrounding southwestern Oklahoma with populations on multiple sides from which invasions could occur (Peper et al., 2017). The discovery of *A. aegypti* is important due to its disease transmission potential and risk in Oklahoma where control programs are limited and outbreak protocols may not be up to date.

Container-breeding species can become the source of significant outbreak of arboviruses due to the sequestering of breeding sites and blood-meal hosts in urban areas. Because of the limited understanding of how these three main species of contain-breeding mosquitoes interact within urban areas in Oklahoma, a new region for *A. aegypti* in the United States, the aim of the

study was to begin examining the ecology and potential risk that container-breeding mosquitoes pose in the southern Great Plains. To accomplish this, three objectives were developed:

1.) Determine *Aedes aegypti* and *Aedes albopictus* distribution in urban areas in central and western Oklahoma.

2.) Identify prevalence of *Dirofilaria immitis* in container-breeding mosquito species collected in urban areas of Southern Oklahoma.

3.) Identify predictive variables for container-breeding species distribution in urban areas using habitat modeling.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Since the expansion to the new world, people have been encroaching on native habitats. This new distribution causes native mosquito species to either vacate their original niches or adapt (Powell and Tabachnick, 2013). Adaptation to a new habitat closely related to human dwellings can be referred to as domestication as certain mosquito species have become 'tame' in the respect of living inside human dwellings instead of the outdoors (Powell and Tabachnick., 2013). Indoor mosquito species are often known as container-breeders due to ovipositioning in flower vases, bird-baths, old tires, and other containers that collect water for a period of time (Simoy et al., 2015). Container-breeding species are commonly from the mosquito species that have evolved to consume blood from the most available and stable sources in their immediate environment: humans (Powell and Tabachnick, 2013). This specificity to humans has caused an increase in the transmission of certain arboviruses around the world.

History of *Aedes* species in the United States

One of the best-known container-breeding mosquito species in the world is *Aedes aegypti.* Although commonly known as the yellow fever mosquito, *A. aegypti* emerge

from an ancestral form of *A. aegypti formosus* with a distant relative still residing in the forests of sub-Saharan Africa (Powell and Tabachnick, 2013). *Aedes aegypti formosus* uses tree holes for larval habitats, while primarily feeding on non-human mammals (Powell and Tabachnick, 2013). *A. aegypti aegypti,* on the other hand, uses containers around human habitation for larval habitats and feeds primarily on humans (Powel and Tabachnick, 2013). The *A. aegypti,* commonly found in the U.S. and other parts of South America, is a domesticated form of *A. aegypti formosus* (Powell and Tabachnick, 2013).

A likely scenario of the domestication of *A. aegpyti* in the United States is that *A. formosus* was native to North Africa 6,000 years prior to the formation of the Saharan desert when it was a green, vegetative environment due to wetter climate (Claussen et al., 1997; Powell and Tabachnick, 2013). It appears that as the water dried in North Africa, *A. aegypti formosus* became isolated away from the original population (Powell and Tabachnick, 2013) and adapted to human dwellings where water availability was more abundant. This genetically isolated small population of *A. aegypti formosus* evolved into today's *A. aegypti aegypti* species that is now one of the most significant arbovirus vectors around the globe. *Aedes aegypti* most likely spread to the Americas as early as the 1500's with European expansion and the slave trade, as the ships contained humans to feed on and containers in which to breed (Slosek, 1986). The species had already established in Mexico (Yucatan) around 1648 when there was an outbreak of Yellow Fever (Tabachnick, 1991). As of 1964, *A. aegypti* was still prominent in the southeastern United States, inhabiting 10 states (Morlan and Tinker, 1965; Hahn et al., 2016). Between 1995 and 2016, a collection of surveillance data was compiled to update the current populations of *A. aegypti* in the United States (Hahn et al., 2016). During the summer of 2016, *A. aegypti* was collected in 26 states, mainly in the southwest and along the east coast (Hahn et al., 2017).

Another important container-breeding vector found throughout the southern United States is *A. albopictus.* This species is native to Asia where it took on the name 'the Asian tiger mosquito'. Sometime in 1985, *A. albopictus* established a breeding population in a pile of imported tires in Texas (Sprenger and Wuithiranyagool, 1986). Although, *A. albopictus* was reported earlier in the United States (Pratt et al., 1946), it was confirmed as established only in 1985 (Sprenger and Wuithiranyagool, 1986). The spread of *A. albopictus* throughout the southeast US occurred rapidly, while it took longer to establish in the warmer areas of the west and colder areas of the north (Moore, 1999). By 2016, *A. albopictus* had dispersed to 40 different states in a vast diversity of climatic ranges and habitats (Hahn et al., 2017)

History of *A. aegypti* and *A. albopictus* in Oklahoma

Evaluation of diversity of mosquito populations in Oklahoma began in 1940, concluding the presence of 40 species (Rozeboom, 1942). This number increased to 62 in subsequent surveys in the 1960's and 2000's (Noden et al., 2015b). Through a series of surveys between 1990 and 2004, *A. albopictus* was collected in 69 of 77 counties (Noden et al., 2015a). A competitor with *A. aegypti, A. albopictus* was recorded in Oklahoma during the early 1990s (Moore, 1999) but no *A. aegypti* was collected in any of the subsequent surveys (Noden et al., 2015b). Although present in Oklahoma in the late 1930's, even as far north as Stillwater, *A. aegypti* was only confirmed in 2015 when collected in four southern cities (Bradt, 2017). In Florida, research has shown that that distribution of *A. aegypti* significantly decreased with the invasion of *A. albopictus* (O'Meara et al., 1995). It is not clear what the relationship might be between two important *Aedes* vectors in Oklahoma as *A. aegypti* was eliminated from the state by the of the 1960's (Noden et al., 2015b), which is 20 years before the invasion of *A. albopictus*. While there is a need for increased surveillance for *A. aegypti* across the southern United States, a focused effort to understand the interaction of these *Aedes* species within the southern Great Plains could provide important clues on which to base future control and management strategies to avoid future arboviral outbreaks.

Interactions of *Aedes* species in the United States

Since the early 1980's, *A. aegypti* populations in Florida have decreased in abundance and habitat range due to an increase of *A. albopictus* (O'Meara et al., 1995). This change in local distribution of *A. aegypti* may have affected the arbovirus transmission risk due to having a different vector competence than *A. albopictus* (Simard et al., 2005). In Florida where both species have continuously interacted for decades, *A. aegypti* prefer urban habitats, while *A. albopictus* often resides in more rural or forested area (Reiskind and Lounibos, 2013). The dominate presence of *A. albopictus* inhabiting in rural areas appears to have caused *A. aegypti* to be geographically restricted to more urban areas (Reiskind and Lounibos, 2013). Often, researchers refer to these dry urbanized areas as a refuge for *A. aegypti* away from *A. albopictus* invaders (Hopperstad and Reiskind, 2014).

These two important mosquito vectors are found in areas with distinct ecological conditions in the Florida ecosystem. Because of its ability to withstand desiccation better than *A. albopictus*, *A. aegypti* disperse to areas with hotter temperatures and lower levels of humidity (Reiskind and Lounibos, 2013). Conversely, *A. albopictus* will establish in areas with cooler and wetter terrain (Reiskind and Lounibos, 2013). This seasonal difference may be due to more availability of *A. aegypti* eggs to survive during the dry season while *A. albopictus* repopulates later in the wet season and becomes more abundant (Reiskind and Lounibos, 2013). Even though *A. aegypti* often try to keep their larval habitats away from *A. albopictus*, competitive reduction between the two species is still common (Reiskind and Lounibos, 2013). There is a need to identify whether these same relationships occur between these two important species in other areas of the United States that lack such ecological conditions. Areas of the southern Great Plains, such as Oklahoma, where cities have far less impervious surface, rainfall, and humidity may not have the same distribution and interactions between *A. aegypti* and *A. albopictus.*

Competitors with Container breeding *Aedes* Species

The two most common urban container-breeding competitors of *A. aegypti* are *A. albopictus,* and *C. pipiens complex L.* (Costanzo et al., 2005). *A. albopictus* commonly shares larval habitats with *A. aegypti* (Braks et al., 2003). While *C. pipiens* competes mainly against *A. albopictus* for larval resources (Costanzo et al., 2005), *A. albopictus* is not the only species to invade the United States as *C. pipiens* is also on the rise in the urban areas (Fonseca et al., 2004). *C. pipiens* invaded the U. S. in the early 1800s and is considered a naturalized species due to its long history and wide distribution (Say, 1832; Mori et al., 2007). Within the *Culex* complex, *C. pipiens pipiens* and *C. pipiens quinquefasciatus,* together forming the *C. pipiens* complex (Fonseca et al., 2004; Savage et al., 2006; Harbach, 2012), are the most commonly found species within the homes of permanent residents alongside the *Aedes* species (Fonseca et al., 2004). *Culex pipiens* was thought to have originated from the Americas, but Harbach (2012) confirmed the species came from Africa. Both sub-species are distributed in the United States from as far east as Florida to as far as southern California (Barr, 1967; Andreadis, 2012). *C. pipiens* is known as the northern house mosquito, while *C. quinquefasciatus* is known as the southern house mosquito *(*Say, 1823*). Culex quinquefascuatus* can be found between latitudes south and north of 36°N, while *C. pipiens pipiens* stays above the 39°N (Savage et al., 2006). In the case when these three species of container-breeding mosquitoes are interacting, *A. albopictus* commonly shares larval habitats with *A. aegypti* (Braks et al., 2003), while *C. pipiens* competes against *A. albopictus* for larval resources (Costanzo et al., 2005).

***Aedes aegypti* Life Cycle**

The life cycle of *A. aegypti* revolves around the laying of eggs in suitable conditions involving abiotic factors such as rain, humidity, and temperature (Simoy et al., 2015). In the United States, *A. aegypti* has climate limitations in its ability to spread throughout the country.

The upper limit is the January isotherm of 1.8°C in the northern United States (Monaghan et al., 2018), while the southern limit is around 10°C in the July isotherm (Christopher, 1960; Monaghan et al., 2018). A 10°C isotherm range is common for southern and northern hemispheres during certain seasons (Christopher, 1960; Monaghan et al., 2018). This wide variability of climate ranges can easily affect how *A. aegypti* may disperse throughout a country and continent (Simoy et al., 2015). *Aedes aegypti* often like areas of dry climate within abundant urban environments where humans supply a water-enhanced habitat, e.g., through watering lawn vegetation (Monaghan et al., 2018). These urban *A. aegypti* oviposition sites provide an environment in which eggs lay dormant until fully submerged in water. *A. aegypti* has been observed to oviposit above the water line in human containers such as flower vases, water containers, water bottles, and old tires (Simoy et al., 2015) Their eggs escape desiccation and can be dormant for up to a year in warmer winter conditions (Simoy et al., 2015).

Once flooded with water, *A. aegypti* eggs hatch within 24 hours. The time of hatching may be increased or delayed if the egg senses other factors such as low temperatures or drought. Once hatched, the first instar will emerge and swim to the surface to obtain oxygen before scavenging for a nutritious food source (Zettel and Kaufman, 2016). The larvae feed on bacteria, yeast, and other types of organic matter found in the aquatic environment (Fay, 1964). Larvae will continue to molt or grow through a series of four instar stages over a seven-day period (Zettel and Kaufman, 2016). The 4th instar larvae will begin pupation and complete metamorphosis within 2 to 3 days (Zettel and Kaufman, 2016). This period of metamorphosis can take up to a week depending on temperature ranges (Simoy et al., 2015). Eclosion from the pupae takes roughly 12-24 hours (Zettel and Kaufman, 2016) with about 83% of whole, emerged adults surviving the process (Southwood et al., 1972).

***Aedes albopictus* Larval Life Cycle**

Aedes albopictus also oviposits above the water line of small water-filled containers (Benedict et al., 2007). With the quickest development from egg to pupae in just 7 days at higher temperatures such as 32 ºC, *A. albopictus* pupae can take 1 to 3.5 days at 32ºC before emergence as adults. *A. albopictus* raised at low temperatures had longer larval photoperiods which increased body size (Breigel and Timmerman, 2001) and could be positively correlated with increased protein in larval dietary conditions (Leisnham and Juliano, 2010).

Culex pipiens **Larval Life Cycle**

While *C. pipiens* often uses the same types of containers to oviposition, *Culex* species lay floating rafts of about 100 eggs on top of the water (Hill and Connelly, 2016). It will only take the eggs a little over a day to fully hatch into first instar larvae (Hill and Connelly, 2016). *Culex pipiens* live on different nutrient material than *Aedes* such as organic, industrial pollution in contaminated water (Costanzo et al., 2005). *Culex pipiens* larvae mature in about a week at 30 ºC, which is a little shorter than that of the *Aedes sp.* (Hill and Connelly, 2016). However, in an environmental setting, this temperature condition may be elevated or lowered due to other factors (Mori et al., 2007). The *Culex* sp., however, are similar to *Aedes sp*. in that they have four stages of larvae before transforming into pupae (Hill and Connelly, 2016). Once the larvae stage is complete, the pupae will continue to grow in the aquatic habitat for another day or two at a temperature of at least 27ºC until adult emergence. As female selection of oviposition sites is crucial for survival offspring, understanding site selection of mosquitoes may help researchers reduce vector population through control programs.

Container Species Oviposition Site Selection

Mosquito distribution is often limited to where the female can lay her eggs and the eggs can reasonably survive. Female mosquitoes often use visual or olfactory ques to the select site of best fit for their eggs. Once a site is found, she will test the water by using the hair on the pads of

her feet to determine if the water is the correct quality (Navarro et al., 2003). *Aedes* mosquitoes often pick sites of low salinity and acidity but high bacterial composition (Navarro et al., 2003). Bacterial composition, particularly with container habitats, is highly correlated with *Aedes* abundance (Nilsson et al., 2018). The bacterial communities are also Genus specific, where *Aedes sp*. typically are more abundant when specific forms of physiochemical parameters are correct (Nilsson et al., 2018).

Competition between *Aedes sp.* in containers may cause the decline of *A. aegypti* in larval habitats due to the invasion of *A. albopictus* (Juliano, 1998). The cause of the decline of *A. aegypti* may depend on whether *A. albopicus* is established (sympatric) prior to *A. aegypti* with *A. albopictus* most often yielding more larvae than *A. aegypti* (Leisnham and Juliano, 2010; Wong et al., 2011). While both species are known container-breeders and can inhabit niches in close proximity to humans, their unique ability to survive and grow on specific detritus is dissimilar (Murrell and Juliano, 2008). These differences in food source for larvae may influence the ovi-positioning female to find a good site for her offspring (Nilsson et al., 2018). Sites dominated by *A. aegypti* often have increased abundance of grass detritus (high quality) in urban areas while sites with *A. albopictus* uses non-nutritious sources such as pine needles or low quality detritus (Murrell and Juliano, 2008).

Another aspect of site selection by *A. aegypti* that may influence oviposition involves visual cues (Bentley and Day, 1989). A comparison of the attractiveness to GAT traps by *A. aegypti* and *A. albopictus* to BG Sentinel traps in a field setting concluded that size and color significantly influenced collection rates (Ritchie et al., 2014; Johnson et al., 2017). Selection factors involved with oviposition sites may influence the success or failure of a species during competition as larvae.

Competition and Feeding Behavior of Aedes Larvae

Along with the invasion of *A. albopictus* in the U.S., Moore and Mitchell (1997) reported a rapid decrease of *A. aegypti* populations in certain mixed species larval habitats but not in single species sites. This displacement of *A. aegypti* was possibly caused by the level of competition between the larvae of the two container breeding species competing for nutrient resources (Juliano, 1998). While both species can survive off leaf litter, *A. aegypti* is a superior competitor when the organic material consists of animal detritus (Barerra, 1996). *Aedes albopictus* can be the superior competitor when food quality is low but abundant (Juliano, 2010), but *A. aegypti* is the more efficient competitor when food is high quality and abundant.

Along with competition for food resources and habitat, *A. albopictus* is a superior competitor with the ability to reduce *A. aegypti* fecundity. This is called mating inference in where *A. albopictus* males mate with *A. aegypti* female causing sterilization of the female and may explain for the displacement of *A. aegypti* by the invasion of *A. albopictus* in certain areas of the United States (Bargielowski et al., 2015).

Competition of *Aedes albopictus* vs. *Culex pipiens*

As mentioned earlier, another competitor among these container-breeding *Aedes* species is *Culex pipiens* complex *L*. *Culex pipiens* often compete with *A. albopictus* during the larval stage in water-filled containers commonly co-occurring in old tire sites within urban residential areas (Costanzo et al., 2007). In these instances, *A. albopictus* is often a superior competitor when resources are limited, while *Culex pipiens* complex L. can also survive these harsh conditions (Costanzo et al., 2007). *Aedes albopictus* can survive in resource-poor condition by converting limited amounts of food into large quantities of biomass, providing a superior edge in development time for larvae (Carrieri et al., 2003). *Culex pipiens* complex also had survivorship at the high density treatment as well as the low density. The competition between *C. pipiens* and

A. albopictus is highly asymmetrical, allowing *A. albopictus* to be superior among container-breeding species (Costanzo et al., 2007).

Trapping Methods

For effective surveillance of potential mosquitoes involved in arbovirus transmission, determining which particular species of mosquito one desires to collect is crucial before trap selection. Mosquitoes are diverse in their habitat and niche specification with some laying eggs in areas of floodwater while others using natural or artificial containers. Depending on the species of interest, different traps may influence collection rates. For container-breeding species in competition for habitat and nutrient resources, several trapping methods are available to enhance the knowledge of mosquito communities in a given area. The most common trapping methods are CDC light traps, commercial propane traps, BG sentinel traps, and gravid Aedes traps (GAT) (Hoel et al., 2009; Johnson et al., 2017). When attempting to collect a variety of species, CDC light traps with either commercial propane traps or containers with dry ice are best. While CDC light traps commonly collect a general variety of mosquitoes in a given area, especially *C. pipiens* complex (Cilek et al., 2017), GAT and BG sentinel traps target more container-breeding *Aedes* species (Farajollahi et al., 2009; Johnson et al., 2017). Once collected, specimens can be identified and tested for pathogens, or if alive, the mosquitoes can be used for behavior or physiology studies of individual species. While usually used for research purposes to better understand the distribution of a species and their potential arboviruses, community groups have recently started using these traps to reduce mosquito populations in urban areas in conjunction with their citizen science programs (Bazin and Williams, 2018).

Pathogen Transmission of Container Breeding Species

Vector-borne diseases affect undeveloped countries as well as developed countries such as the United States. Throughout the history of the United States, mosquitoes caused outbreaks

of vector borne pathogens such as Yellow Fever, Malaria, West Nile, and filaria (Gubler et al., 2001). While the U.S. has successfully decreased or eliminated the transmission of diseases through extensive vector control programs and changes in human behaviors, new arboviruses continue to threaten (Gubler et al., 2001). The latest mosquito-borne diseases of concern to the U.S. are Zika, Dengue, and Chikungunya viruses (Gubler et al., 2001). In order for disease to spread efficiently, components such as host, vector, and pathogen must all be in the same location within a given landscape ('nidus' of infection) (Reiskind et al., 2016). Because of this, all vector-borne disease transmission varies depending upon vector abundance, seasonal distribution, habitat and host preference (Day, 2005; Reiskind et al., 2016).

Canine Heartworm

One of the prevalent pathogens which impact companion animals in the United States is canine heartworm caused by *Dirofilaria immitis* (Ledesma and Harrington, 2011). *Dirofilaria immitis* has a complex reproduction cycle, starting in infected canines, domestic or wild. The dogs are fed upon by a competent vector in which L3 stages of the worm have developed (CDC, 2018a). The L3 stages use the wound produced by the mosquito bite to enter into the host and infect the canine's muscle tissue. Once the L3 larvae have become young adults, the worms migrate to the pulmonary arteries to mature into their sexual reproduction stages (Ledesma and Harrington, 2011). Fully mature worms produce offspring known as microfilaria which spread through the blood stream and are ingested by feeding mosquitoes (CDC, 2018a). In the mosquito, the microfilaria migrates to the Malpighian tubules of the mosquito to form the L2 stage (CDC, 2018a). Once large enough, the worms travel through mosquitoes' hemocoel toward the head. The final stage of L3 development occurs on the entrance into the head allowing an infective worm to be present upon feeding when it breaks out of the palps onto the skin of the animal (Ledesma and Harrington, 2011).

Dirofilaria immitis or canine heartworm has been found in the domestic dog for nearly 400 years with the first reports occurring in Italy (Lee et al., 2010). Of the sixty-three known vectors of *D. immitis,* twenty-eight competent species have been identified in the U.S. (Licitra et al., 2010). The two principal vectors for *D. immitis* in the United States are *A. albopictus and Culex pipiens* complex L. *(*Ledesma and Harrington, 2011). With the spread of *A. albopictus*, the risk for this disease has increased in local urban communities like those in Oklahoma (Paras et al., 2014). While canine heartworm mainly infects domestic dogs, other wild canines such as coyotes, foxes, and wolves have been identified as reservoirs (Lee et al., 2010). Adult worms have been detected in other vertebrates such as humans, cats, sea lions and horses, but the infective stage, microfilaria, has not been recovered in these dead-end hosts (Lee et al., 2010). Although domestic cats are also a dead end host, *D. immitis* is particularly deadly for these animals (Litster et al., 2008).

Zika Virus

In 1947, researchers discovered a flavivirus in primates in Uganda and named it Zika virus. This flavivirus is similar to Yellow Fever, Dengue, and West Nile viruses (Campos et al., 2015). Until 2007, this virus was isolated to equatorial areas of Asia and Africa (Monaghan et al., 2016). However, with an explosive expansion of Zika to the Yap island in French Polynesia then into Brazil, concerns increased in the United States that local outbreaks could occur within local *Aedes sp*. after feeding on infected travelers (Monaghan et al., 2016; Hahn et al., 2016).

Coincident with this concern for an outbreak in the U.S. was the discovery of more *A. aegypti* populations in different communities (Monaghan et al., 2016). Possible transmission of Zika virus is increased during summer months and in areas with high human population in low socioeconomic conditions (Monaghan et al., 2016). Zika,

although known to cause rash, fever, arthralgia, and conjunctivitis, can also cause severe fetal birth defects such as microcephaly. Not only can it be transmitted via the bite of a mosquito, it is also transmitted through sexual intercourse (Musso et al., 2015). Another aspect of concern caused by Zika virus is the increase of local cases of the autoimmune disease, Guillain-Barré syndrome (GBS), with reports in 15 countries with increased symptoms of Zika and individuals expressing GBS (WHO, 2016). GBS is a neurological condition where an individuals' immune system attacks the peripheral nervous system. However, according the National Institute of Neurological Disorders and Stroke (2018), individuals can recover from GBS over time unlike microcephaly.

Yellow Fever

Yellow fever, a viral hemorrhagic fever, is a caused by another Flavivirus and primarily transmitted through *Aedes sp*. mosquitoes (CDC, 2018c). The virus can be mild to severe causing liver disease and jaundice (CDC, 2018c). Yellow fever is commonly found in a sylvatic cycle involving primate reservoirs and the mosquitoes which feed on them. The disease moves to cities often when people living close to the sylvatic settings are fed upon by infected mosquitoes and bring the infection into the urban setting where *A. aegypti* is present (CDC, 2018c). The transmission cycle of most concern, especially for the United States, is the urban cycle where the virus is spread human to human via domestic *Aedes sp*., such as *A. aegypti* (CDC, 2018c). While Yellow Fever was historically a problem in the United States, the use of rigorous control effects and more efficient / secure housing conditions eradicated it in the early 1900's (Gubler, 2004). The reduced threat of Yellow Fever in the U.S. provided the opportunity to invest resources in other regions such as South America where control and elimination efforts continued until around 1957. Another aspect of the eradication of yellow fever from the United States and most of the world was the production of a vaccine in 1928. This vaccine is a weakened live form of the virus,

that is safe and efficacious, protecting individuals for up to ten years or longer with reports of individuals with antibodies up to 40 years (WHO, 1991).

Dengue

As the risk of yellow fever decreased, other viral pathogens emerged to became important mosquito arboviruses on a global scale. Dengue virus or Dengue hemorrhagic virus has been identified globally since the 1800's due to the transport of infected mosquitoes via the shipping industry and movement of people (Gubler, 2002). While global in its reach, Dengue did not appear as a significant problem until the 1950s (CDC, 2018d). While occasional cases occur, the United States is not endemic for Dengue, although it occurs in countries on our borders as Puerto Rico and various countries of South America (CDC, 2018d). Unlike Yellow Fever and Zika virus, Dengue exists as four virus serotypes (Gubler, 2004). Symptoms of a single virus serotype causes "break bone fever" as individuals experience severe muscle and joint pain along with high fever (CDC, 2018d). When Dengue serotypes overlap or are transmitted to an individual simultaneously, infected individuals can experience viral hemorrhagic fever, leading to significant physical discomfort and death. The main mode of transmission of Dengue virus is through the *Aedes* mosquitoes. Like other mosquito arboviruses, Dengue is commonly found in monkey reservoirs in Asia, the South Pacific, and Africa. However, within endemic areas in Asia and Africa, infective humans are also contributing to the spread of Dengue through the intermediate bites of *Aedes* mosquitoes (Gubler, 2004).

Chikungunya

Another arbovirus that has exploded on the global stage in recent years is Chikungunya virus. Like other mosquito arboviruses, Chikungunya can easily enter a local population because of increased human travel and the presence of *Aedes* vectors (CDC, 2018e). Currently, there is no

vaccine available. Chikungunya was first identified in 1953 in Tanzania and is now commonly found throughout South America, the Pacific Islands, Asia and parts of Africa. Although not considered endemic, there were several transmitted cases in the United States in 2013, particularly in southern Texas (Hotez, 2018). Most of the cases in United States residents occurred via travelling or working abroad (Lindholm et al., 2017). Chikungunya is not commonly fatal, however, severe discomfort is associated with symptoms of fever, rash and joint pain (Pialoux et al., 2007). Translate from the Makonde language in northern Mozambique, Chikungunya means "to walk bent over" due to the incapacitating joint pain (Pialoux et al., 2007). This virus commonly has outbreaks when vector populations, namely *Aedes aegypti* and *Aedes albopictus,* are abundant *(*Pialoux et al., 2007).

West Nile

The most important arbovirus in the United States in the last 20 years is West Nile Virus. Unlike the other mosquito arboviruses, West Nile, although part of the *Flavivirus* group, is transmitted mainly by *Culex sp.* West Nile emerged in the United States in the late 1990s. By 2003, the virus had spread across most of the continental U. S. although the exact means by which this happened are not well understood (CDC, 2018b). West Nile is commonly found within reservoir bird populations such American robins, doves, house sparrows, and blue jays (Komar et al., 2003; Kilpatrick et al., 2008). This virus can infect mammals such as a humans and horses, but these are considered 'dead end hosts' and do not provide a high enough viremia to be infective to mosquitoes (Kilpatrick et al., 2008). The virus is known to be fatal to many mammalian species, although most individuals do not express symptoms. When infected, West Nile can produce complicated neurological problems in elderly or immunocompromised individuals that can involve fever, headaches, vomiting, and rash, or in severe case neurological effects (CDC, 2018b). While humans are not the typical food source for *Culex sp*. transmitting West Nile, increase in transmission often occurs during drought season when food sources are

limited (Epstein and Defilippo, 2001). Understanding the potential risk during a drought year can help control programs have a reason to maintain waterways and flood areas with urban areas where reservoirs and vectors are abundant (Epstein and Defilippo, 2001).

Geographical Information Systems

Geographical information systems (GIS) are computer based software that allow for creating, acquiring, visualizing, analyzing, and modeling information about the surface and near-surface of the earth (Aitken and Valentine, 2006). GIS can be used to map model and better understand changes in geographic phenomena such as weather, land use, or population density over time or time/space (Aitken and Valentine, 2006). GIS allows researchers to understand relationships in a given environment, while having a visual representation on a spatial scale (UOW, 2018). The use of GIS in vector ecology can help emphasize the variability of the environment that may influence mosquito populations and represent areas of potential outbreaks (Kolivras, 2008).

CHAPTER III

Distribution of Container-Breeding Mosquitoes in Urban Areas of Southern Oklahoma

Abstract

Aedes aegypti, the yellow fever mosquito, is a significant arbovirus vector worldwide and one that has gained prominence recently in the US as a primary vector for Zika virus. In 2016, *A. aegypti* was discovered again in four cities in southern Oklahoma during surveillance activities along with other important container-breeding species, namely *Aedes albopictus* and *Culex pipiens* complex. While pockets of *A. aegypti* in several Oklahoma cities were identified, there is limited understanding of the nature and extent of these populations within given urban areas or regions of the state. In this study, we hypothesized that *A. aegypti* were more likely to occur in the southern part of the state and were more likely become established within regional urban areas. Between May to August 2017, mosquitoes were collected in six urban areas along two transects in central and western Oklahoma between the Red River (Texas border) and cities 60 miles from the border.

.Bi-weekly mosquito collection utilized Gravid Aedes traps (GAT) and BG-sentinel traps across urban gradients. With the use of geographical information systems (GIS), predictions of mosquito density in relation to vegetation, container availability, and other anthropogenic factors were determined within urban habitats. Of the 6,628 female mosquitoes collected, 80% were container-breeding species (*A. albopictus* and *A. aegypti*) with proportions differing between different urban areas. *Aedes aegypti* was more localized in southern Oklahoma, while other container species were more widely distributed. While the prevalence of *D. immitis* in *A. albopictus* and *C. pipiens* complex was low, regression models confirmed significant predictive parameters for container-breeding mosquito species. The results of this study will assist in the prediction of mosquito vector habitat in urban areas of Oklahoma and potentially demonstrate how arboviruses could affect these cities in the event of an outbreak.

INTRODUCTION

Mosquito-borne arboviruses, mainly yellow fever, were a problem in the U.S. for the first centuries and caused the expanding population to develop complex systems to control mosquitoes in order to limit the extent to which these arboviruses affected our health. In the early 1900s, the U.S. enforced an eradication program throughout areas where *Aedes aegypti*, the main mosquito vector, was present. These eradication efforts dramatically reduced vector abundance which, in turn, reduced the risk of contracting Yellow Fever. Although small outbreaks of arboviruses continued to occur, the success of these eradication programs ended in 1999 with an epidemic of West Nile virus that swept across the US, starting in New York and ending in California in 2003 (CDC, 2018b). To date, West Nile virus continues to be endemic throughout the United States. Recent

outbreaks of chikungunya and Zika virus in the southern Americas region with the continued threat of Dengue coming into the country via persons travelling to regions experiencing outbreaks or infectious people moving into the US continue to emphasize the need to be vigilant. This increased need for vigilance correlates with an accompanying need to identify where specific competent vectors, specifically, *Aedes* container-breeders, are thriving in local landscapes. The main container breeders in the United States that impact the spread of arboviruses are *Aedes aegypti*, *Aedes albopictus* and *Culex pipiens* complex L.

Aedes albopictus and *C. pipiens* develop quickly in the right conditions and are widely distributed across the southern United States while *A. aegypti* is established in more localized areas such as Florida, Arizona, Texas, and California (Hahn et al., 2017). While these populations are characterized, their ecology is not well understood in the Great Plains region. In Oklahoma, *A. albopictus* was identified in all 11 eco-zones, which demonstrated the ecological flexibility of this container-breeding species (Noden et al., 2015a). *Culex pipiens* was also reported across the state, but is more localized in the east and central part of Oklahoma (Noden et al., 2015b; Bradt, 2017). In 2016, this scenario was enhanced when *A. aegypti* adults were collected and identified in southern Oklahoma for the first time since the 1940's (Bradt, 2017).

The discovery of *A. aegypti* in Oklahoma lead to many questions as the establishment of the species in Oklahoma may have occurred by multiple introductions from neighboring states or wind-blown populations from Texas. At the time of discovery, surveillance activities in Texas confirmed that *A. aegypti* populations were present in most of the counties along the Red River or the Texas border (Hahn et al.,

2017), which correlated with Oklahoma counties where *A. aegypti* were found (Bradt et al., 2017). *Aedes aegypti* was also discovered along the eestern state border of the Texas panhandle, surrounding southwestern Oklahoma with populations on multiple sides from which invasions could occur (Peper et al., 2017). The discovery of *A. aegypti* is important due to its disease transmission potential and risk in Oklahoma where control programs are limited and outbreak protocols may not be up to date.

Container-breeding species can become the source of significant outbreak of arboviruses due to the sequestering of breeding sites and blood-meal hosts in urban areas. Because of the limited understanding of how these three main species of contain-breeding mosquitoes interact within urban areas in Oklahoma, a new region for *A. aegypti* in the United States, the aim of the study was to begin examining the ecology and potential risk that container-breeding mosquitoes pose in the southern Great Plains, particularly in small urban areas.

METHODS AND MATERIALS

Study Area: The urban areas chosen for this study were based on results from a 2015 mosquito survey conducted in six urban areas in Oklahoma in which *A. aegypti* was discovered in the southwestern and southcentral regions of the state (Bradt, 2017). Although both regions were north of the Texas border, they differed climatically due to longitudinal differences (Anon, 2018). To focus on these unique regions, latitudinal transects were selected prior to mosquito trapping using Google Earth imaging (Google Earth Pro, Google Inc., Mountain View, CA). Each regional transect involved cities from the Texas border along the Red river due to the reports of *A. aegypti* in most of the

counties on the Texas side of the river (Hahn et al., 2017). The south central transect consisted of urban areas located along Interstate 35 traveling north from the Texas border

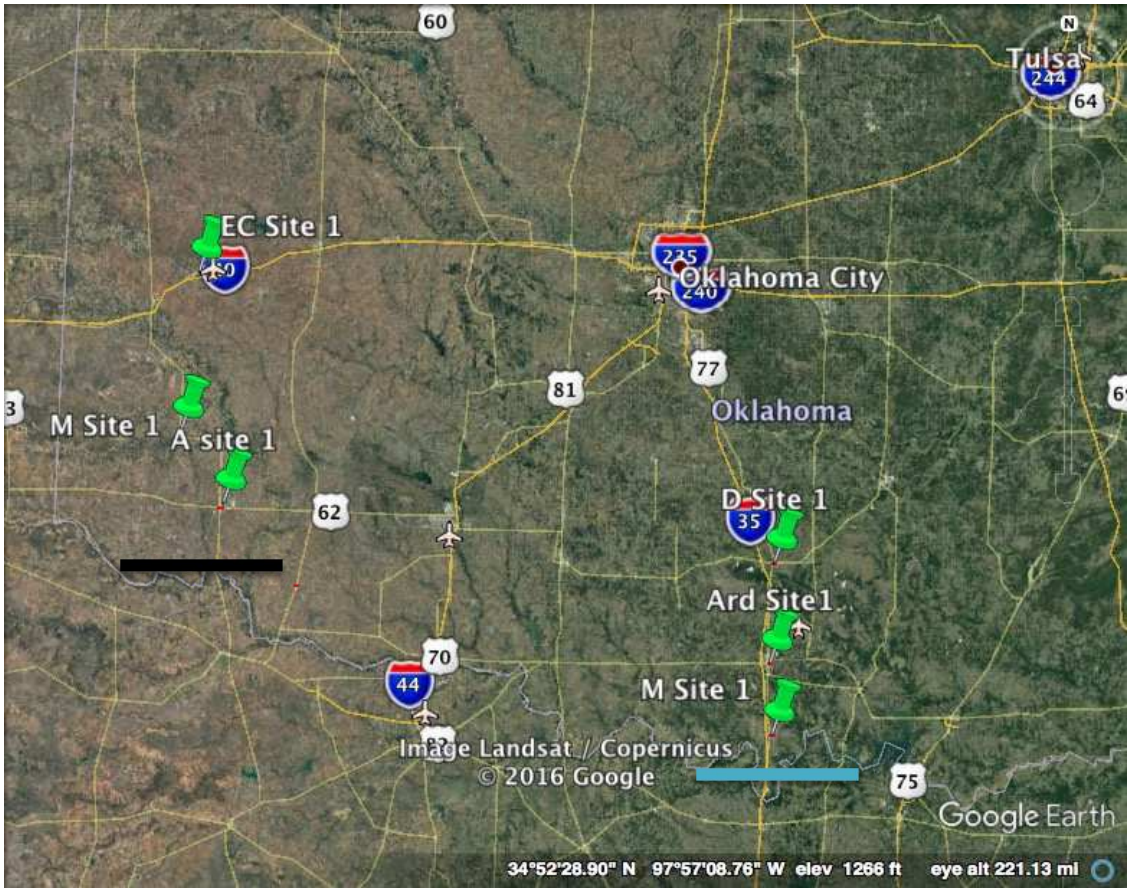


Figure 1: Mosquito Trapping Transects in six Oklahoma cities during Summer 2017. Central Transect underlined in blue and Western Transect in black.

(Figure 1). Cities in this transect were deliberately chosen around the *Aedes aegypti*-positive site of Ardmore (Bradt, 2017). The urban sites Marietta, Ardmore, and Davis were chosen because they have large enough urban areas in which to place transects of traps in local communities. Marietta (elevation 850') is located 25.8 kilometers north of the Texas border, while Ardmore (elevation 875') is located 51.5 kilometers north of the Texas border. Davis (elevation 848') is located 88.5 kilometers north of the Texas border (Google Earth Pro).

The cities (Altus, Mangum, and Elk City) used for the southwest transect were chosen around the *A. aegypti*-positive site of Altus (Figure 1) (Bradt, 2017) and were selected on the basis of having enough urban area to place transects of traps in local communities. Altus (elevation 1402') lies 23.3 kilometers north of Texas border. Mangum (elevation 1603') lies 53.9 kilometers north of Texas border, and Elk City (elevation 1921') lies 112.7 kilometers north of Texas border (Google Earth Pro).

The relative populations of the cities based on the 2010 Oklahoma census (V2017) were: Central transect (N-S): Davis: 2,687, Ardmore: 24, 493, Marietta: 2,628, and western transect (N-S): Elk City: 11, 669, Mangum: 2,991, Altus: 19,831 (Bureau, 2018). Population size was included to reflect the size the urban area considered for the city sites selected. According to the Oklahoma census bureau, towns or cities with less than 50,000 residents are considered to 'urban clusters', while 'urban areas' consist of more than 50,000 residents. Selected sites for this study are therefore considered as 'urban clusters'.

Site Permission Verification: Once regional transect cities were chosen, Oklahoma Cooperative Extension educators were contacted in each county where an urban site was chosen. In collaboration with county extension educators, meetings were scheduled with city officials and members of the local police to discuss the objectives of the study which included the public health risks of *Aedes* species within urban areas, identify site specific methods of informing communities that Oklahoma State University (OSU) students would be approaching residents, and confirm the safety of specific trap

sites chosen within each urban area. Depending on each city, local newspaper articles or Facebook media were utilized two weeks prior to initiating collections to inform the public of the mosquito survey being conducted throughout the summer of 2017. Upon approval from the mayor and chief of police, research personnel explored and confirmed possible sites in individual cities using Google Earth imaging. Sites were evaluated based on safety for research personnel and exact site locations were confirmed based on potential mosquito habitat such as vegetation and container availability. Once appropriate trap sites were selected, research personnel contacted each resident or industry at each site to personally explain the rationale and procedure behind the mosquito survey. To reduce time of explaining the specific of *Aedes* species biology and ecology, individuals were provided a brochure (Appendix 1). Each resident or industrial property owner authorized mosquito trap placement in the front area of their property through verbal agreement.

Mosquito Sampling: Two trapping methods were used to establish *Aedes sp.* distribution in the urban cores of the cities selected and the surrounding areas outside of the city center. A systematic trapping method using gravid *Aede*s traps (GAT) (Biogents, Regensburg, Germany) evaluated the distribution of *Aedes* species within urban cores. The surrounding residential areas or outer city limits, outside of the urban core, were sampled randomly using BG-sentinel traps (Biogents, Regensburg, Germany). The purpose of these traps was to evaluate the extent to which various *Aedes sp.* were distributed in the areas outside of the urban core of a particular city. Differing site types

within the core and outer city limits were labelled ‘residential’ or ‘industrial’. Traps were placed in areas of small businesses, town halls, and residential areas.

‘Rural’ sites consisted of a home on the outer limits surrounded by open fields and not in a neighborhood. ‘Agricultural’ sites were located in an area of open fields such as crops with no home or business in direct sight. Using raster GIS datasets provided by the Multi-resolution Land Characteristics Consortium (MLRC.gov, 2018), the percentage of urban or impervious surfaces and vegetation cover were noted during site selection. Due to container-breeding mosquito resting behavior, vegetation coverage around a site was assessed based on scale parameters, mentioned elsewhere in the methods.

Within each city, five 1000-meter transects were plotted using Google Earth imaging. Transects were located at least 200m apart to maximize mosquito population detection in the urban core (Paras et al., 2014; Hopperstad and Reiskind, 2016). An example (Altus) demonstrates the transects in urban cities with transect designs (Fig 2). Appendix 2 shows the layouts of all the trapping sites and transects in all six cities.

Transect sites were sampled with gravid *Aedes* traps from Biogents (Biogents, Regensburg, Germany). Four gravid *Aedes* traps (GAT) were placed on each transect (20 traps/urban area) approximately 250m apart from one another (Appendix 2). This 250m distance was chosen to ensure that mosquitoes from that particular area were being collected and not from other trap zones as container-breeding mosquitoes normally only fly 50m-100m from their breeding sites (Wetering et al., 2014; Reiskind et al., 2016). The urban core transects were surveyed every two weeks between June 12, 2017 and August 17, 2017. Surveying involved a total of 60 GAT traps (20 per city) on Monday and

collecting them on Thursdays, approximately 72 hours of collection, making for a total of 180 trap nights per regional transect per week (Appendix 3).

At each site where GAT traps were used, the traps were placed in well-shaded areas at the front of the property to eliminate the need to get permissions to go into backyards. This location may have reduced the opportunity to find specific species, but it eliminated the need to obtain an additional level of Institutional Review Board permissions through OSU. Based on the recommendation of Heringer et al. (2016), the inside lining of each GAT trap was initially coated with canola oil instead of insecticides. However, the canola application caused mosquitoes to become stuck in the traps and thus unidentifiable. So, after the first round, a 10% concentrate permethrin (Durvet, Blue springs, MO) was applied by product application standard requirements for a knock down of mosquitoes in the GAT traps. The traps were placed on clear plastic plant saucers (Lowe's, Mooresville, NC) and submerged with water to hinder ant infestations.

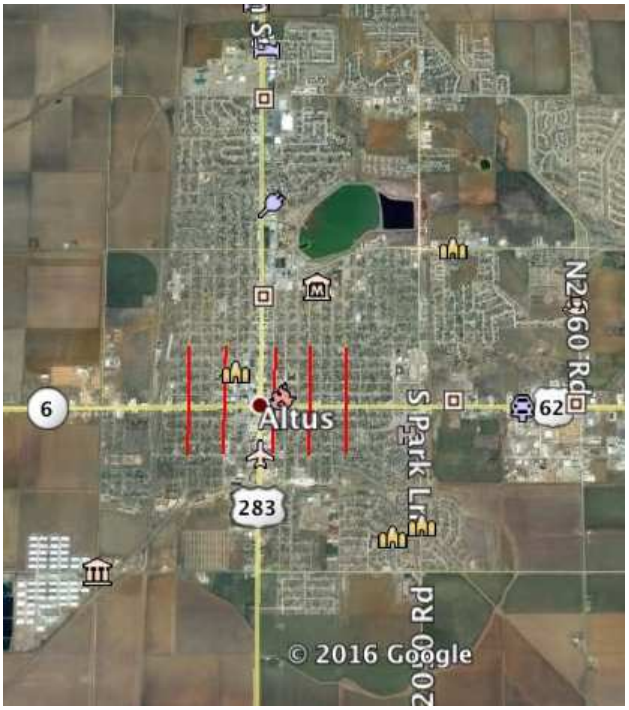


Figure 2. Example of city -1000meter transects

To further augment the trapping in the urban core, 20 additional sampling areas were identified around each urban core for their ecological uniqueness using USGS land cover data together with Google Earth. In the same two week intervals as the urban cores

were surveyed with GAT traps, 10 of these external sites were randomly chosen (Extendoffice.com) and BG Sentinel traps (Biogents, Regensburg, Germany) with BG lure were placed at each site for 20 hours, beginning at noon until 8-9am the next day (Appendix 4). This trap is primarily focused on local *Aedes sp*. and served as an additional method to determine populations within the wider area around each urban core.

Mosquito Identification: Mosquitoes were removed from the traps as soon as possible on a weekly basis with the use of microdissection forceps, placed into 7 dram vials, and stored in a Whynter 45-quart portable freezer (Whynter, Brea, CA) at 20^{o}C prior to identification under a Labomed Luxeo 4Z dissecting microscope (Labomed Inc., Los Angeles, CA). Using Darsie and Ward (2005), each mosquito was identified to species unless unidentifiable due to damage. After identification, all mosquitoes were transferred to -20^{o}C freezers (Frigidaire, Dayton, OH) until further processing. Data on identified mosquitoes were collected using Microsoft excel (Microsoft, Seattle, WA). Due to southern Oklahoma being a hybrid zone, *Culex pipiens* and *Culex quinquefasciatus* were identified as *Culex pipiens* complex L for ease of this study since *C. pipiens* complex L was not the main focus (Harbach, 2012).

***Aedes aegypti* Confirmation Assay:** Samples that were thought to be *A. aegypti* but had the identity markings rubbed off during collection were further tested by dissecting a single leg using sterile tweezers and placing it in a sterile vial. Each vial was labeled with

date, location, and genus of all unknown mosquitoes being processed. The positive control used was *A. aegypti* Liverpool strain continuously reared in the laboratory. One day prior to extraction, using a genomic DNA extraction kit (GeneJET, Genomic DNA Extraction Kit, Thermoscientific, Grand Island, NY), 20µl of ProK and 180ul of Digestion solution were added to each of the sample tubes containing legs of unknown mosquitoes, and each sample was incubated in a shaker overnight at 56ºC. The next day, 200µl of lysis solution and 400µl of 50% ethanol were added to each sample, the samples were vortexed and extraction was completed following the manufacturer protocol. Extracted DNA samples were stored in a freezer at -20ºC for further processing.

The extracted DNA was tested using primers that amplify a 361bp region of the ND4 mosquito gene (Costa et al. 2005): ND4-Forward primer (5'-ATTGCCTAAGG CTCATGTAG-3') and ND4 Reverse (5'- TCGGCTTCCTAGTCGTTCAT- 3'). The initial denaturation step occurred at 94°C for 2 min, followed by 35 cycles at 94°C for 1 min, 56°C for 30 s, and 72°C for 1 min, and a final elongation step at 72°C for 7 min in a Bio-Rad C1000 Touch thermal cycler (BIO-RAD, Hercules, CA). All positive productions were analyzed using agarose gel electrophoresis in 1x TAE buffer with 2% agarose gel stained with ethidium bromide. Gels were examined with an ultraviolet light. All results were photographed and printed for verification and documentation. All positive amplicons detected were sent to Oklahoma State University Core Facility to be bi-directionally sequenced. Resulting sequences were searched using the nucleotide BLAST database to determine the species of mosquito collected.

***Dirofilaria immitis* DNA Extraction:** Known vectors of *Dirofilaria immitis*, *Aedes albopictus* and *Culex pipiens*, were processed for Polymerase Chain Reaction analysis in a sterile lab environment at OSU facilities. As mature *Dirofilaria immitis* are found in the head region of infected mosquitoes, each pool was processed by separating the abdomen using sterile 70% ethanol-cleansed tweezers. Pools of head/thoraces were created using one to ten mosquitoes by trap/site/date. Once mosquitoes were processed, crude DNA extraction occurred by placing the mosquito heads/thoraces into 2 ml sterile polypropylene Sarstedt microvials (Biospec, Bartlesville, OK) with 100 µl of DNAzol (Molecular Research Center, Inc., Cincinnati, OH) then placing the vials into a heating block for 15 minutes at 95ºC. Sterilized zirconia/silica beads (2 large, 6 small) (Biospec, Bartlesville, OK) were added to the heated vials then placed in a Mini-Beadbeater 16 (Biospec, Bartlesville, OK) for two minutes. Upon completion, the vials were centrifuged for 1 minute at 12, 000rpms and the supernatant containing crude DNA was removed and placed into sterile 1.7 ml tubes and frozen at -20°C until PCR analysis.

***Dirofilaria immitis* Detection:** Polymerase chain reaction (PCR) amplification was conducted in a laboratory separate from that used for mosquito processing to reduce potential DNA contamination. Initial PCR screening of all pooled samples was completed using primers COIint-F and COIint-R that amplifies a portion of the filarial mitochondrial DNA cytochromoxidase subunit 1 (COI) gene (Casiraghi et al., 2001). Each 25µl sample contained 12.5µ l GoTaq® Green Master Mix (Promega, Madison, WI), 10.5 lµ DNAse/RNAse free H_2O (Promega), 0.5 µl 5mM COIint-F, and 0.5µ l Mm COIint-R. Addition of 1µl of mosquito supernatant from each pool was added to the reaction vials.

The COIint protocol consisted of a denaturing step at 94 ˚C for 5 minutes followed by 40 cycles of denaturing (94˚C for 45s), annealing (52˚C for 45s), and extension (72˚C for 90s) with a final extension of 74˚C for 7 min in a Bio-Rad C1000 Touch thermal cycler (BIO-RAD, Hercules, CA). For each PCR reaction, a positive control of 0.5 µl of *D. immitis* gDNA was added to a reaction vial as well as a water control reaction vial for a negative control. Samples of *D. immitis* genomic DNA was generously provided by Dr. Rebecca Trout-Fryxell of University of Tennessee and Dr. Michael Reiskind of North Carolina State University. Verification of the positive samples included the use of the *D. immitis* specific primers (DIDR-F1/DIDR-R1), which amplify a region of the internal transcribed spacer (ITS) of the ribosomal DNA (Rishniw et al., 2006). The DIDR-F1/DIDR-R1 PCR procedure consisted of a denaturing step at 94˚C for 5 minutes followed by 32 cycles of denaturing (30 seconds at 94˚C), annealing (30s at 60˚C), extension (30s at 72˚C), a final extension (7 min at 74˚C), and a soak at 4˚C in a Bio-Rad C1000 Touch thermal cycler (Bio-Rad, Hercules, CA). Each positive sample was sequenced to verify pathogen species in each container-breeding mosquito species collected.

All PCR products were analyzed using agarose gel electrophoresis in a 1x TAE buffer with 2% agarose gels stained with ethidium bromide. Gels were examined with an ultraviolet light. All results were photographed and printed for verification and documentation. All positive amplicons detected using the COIint-F1/R1 and DIDR F1/R1 primers were sent to Oklahoma State University Core Facility to be bi-directionally sequenced. Resulting sequences continued to be searched in the nucleotide NCBI-BLAST database to determine the species of filarial detected in the specific

mosquito pool samples. Due to few positives of *D. immitis* from primer sets COIint F1/R1 and DIDR F1/R1, each pool is being retested using a 1:10 dilution of supernatant DNA with DNAase-free water and COIint primers to establish whether there may be components in the crude mosquito extract that might be inhibiting the PCR reaction. Because pools of mosquitoes tested for pathogens were not constant, the minimum infection rate (MIR) was calculated by city for *D. immitis* in *A. albopictus* using the calculation from Condotta et al. (2004) and the CDC Excel Add-in for pooled infection rates (Biggerstaff, 2009).

Variable Field Data Collection: In addition to collecting mosquitoes, other explanatory variables were collected while in the field such as numbers of visible containers, backyard clutter, number of dogs, and percent vegetation. Number of containers per site was calculated by visual assessment from the front yards of homes or businesses as any item that could hold water such as flower vases, bird-baths, and old tires (Simoy et al., 2015). The actual number of containers counted was recorded and put into the dataset. At the same time as container assessment, the number of dogs in the area around a house was calculated by visual assessment throughout the research season with notes taken on which canines were repeatedly present at each resident. Backyard clutter was assessed visually from Google Earth imagery due to privacy limitations. Backyards were categorized with low, medium, or high volume of clutter at each site or surrounding areas. Low clutter was distinguished by mostly vegetation surrounding a site, with one or 2 containers visible. Medium clutter consisted of an area with 10+ containers. High clutter areas were noted with more than 25+, typically areas such an old car salvage yard,

or waste dumps. The percent vegetation was replicated following Walker et al. (2011) and involved an estimation by visual examination by a single viewer for consistency. Sites were labeled in categories of 1 to 4. A site with 0-10% vegetation coverage was considered a level 1 or no_veg, 10-25% a level 2 or low_veg, 25-50% a level 3 or med_veg, and 50-100% a level 4 or high_veg. Again, all data collected were catalogued in a Microsoft excel file along with the mosquito data from each week.

ArcMap GIS Data Collection: Additional explanatory variables were gathered in GIS raster format from the Multi-resolution Land Characteristics Consortium (MRLC), a group of federal agencies that generate environmental, land management, and modeling data for applications (Mrlc.gov, 2018), providing detailed satellite imagery, land cover databases, and other supplementary datasets. For the current study, the National Land Cover Database (NLCD) 2011 USFS tree canopy cover cartographic data and NLCD 2011 developed imperviousness data was obtained from the MRLC website. Both of these datasets are generated based on Landsat imagery and have a spatial resolution of 30m. In each case, individual pixels contain a percentage value for either the proportion of tree cover or proportion of impervious surfaces within the 30x30m pixel. The formation of NLCD impervious surface dataset is a continuation from 2001 data set in which the impervious surface dataset was produced by finding two images and characterizing the different land cover classes between them as image change detection (Xian et al., 2011).

To secure a more accurate impervious surface estimate, nighttime stable light satellite imagery was added to allow for determining urban boundary based on the location, extent, and brightness of nighttime lights (Xian et al., 2011). Determining the most accurate boundary of urban land cover allows for more precise data in other research areas (Xian et al., 2011). Protocols developed from the NLCD 2011 include source data preparation, spectral change detection, land cover change modeling/mapping, impervious generation, and canopy generation (Homer et al., 2015). Overall tree canopy cover was produced by photographic interpretation of National Agricultural Imagery Program aerial imagery in which close to 65,000 images were used to calculate the percent of tree coverage by photo interpretation for the NLCD 2011 (Homer et al., 2015). Two different forms (Analytical and Cartographical) of land cover were produced where the analytical is used to estimate averages of tree canopy cover, while cartographical is used as a visual in cartographical applications. Both datasets allow for better understanding of how landscape change may alter ecological, social, and climatic patterns throughout time.

GIS Methods: Tree canopy cover and developed impervious surface layers were downloaded (MLRC, 2018) and each set of data was initially clipped to the state boundary of Oklahoma using ArcMap. The newly clipped Oklahoma tree cover and impervious surface data was added to ArcMap as new layers. As mentioned in the site selection, site point locations that were initially created in Google Earth were also exported and added in ArcMap as a new layer. The new site points attribute table was joined to an existing Excel sheet consisting of explanatory variables in ArcMap using the

"name" field. The new attribute table for the site points includes the field data information as well as site location data.

Buffers of 100 meter and 250 meters were created around each of the 242 site locations in southern Oklahoma. This 250m distance was chosen to reflect mosquito flight behavior of around 50m-100m, but for good measure, a buffer zone was set for a more accurate representation of mosquito in the area (Hopperstad and Reiskind, 2016). The tree canopy cover raster as well as the developed impervious surfaces area raster were clipped to each set of buffers, creating four datasets per site: canopy cover at 100m and 250m, and urban impervious surface at 100m and 250m. The total amount of tree canopy cover and impervious surface area were aggregated within each buffer, and the resulting value was assigned to the respective site. In total, each site includes thirteen explanatory variables (Table 1): (1) number of containers, (2) number of resident canines, (3) clutter density based on low, med, high scale, (4) percent of vegetation based on a 1-4 scale, (5) total amount of impervious surface within 100m of the site, (6) total amount of impervious surface within 250m of the site, (7) total amount of tree canopy cover within 100m of the site, and (8) total amount of tree canopy cover within 250m of the site. (9) sampling week (10) residential area, (11) industrial area, (12) rural area, and (13) agricultural area.

Table 1. Explanatory Variables collected during summer 2017 for habitat preference analysis

Explanatory Variables	Descriptor
1. #_containers	Number of containers in visibility from site location
2. presence of resident canines	Presence or Absence of resident canine in visibility from site location
3. Clutter Density*	Low, Medium, High
4. Percent Vegetation*	No_veg (1), Low_Veg (2), Med_veg (3), High_veg (4)
5. Urban_100	Total amount of impervious surface within 100m of the site
6. Urban_250	Total amount of impervious surface within 250m of the site
7. Tree_100	Total amount of tree canopy cover within 100 meters of the site
8. Tree_250	Total amount of tree canopy cover within 250 meters of the site
9. Week	Sampling Round
10. Residential	Site location in a neighborhood
11. Industrial	Site location at a business or industrial area
12. Rural Sites	where a home was on the outer limits surrounded by open fields and not in a neighborhood

-Variables exhibiting an (*) are further described in the methods

Statistical Analysis: Mosquito trap sites were analyzed against explanatory variables found in Table 1 and mentioned in the GIS methods. A stepwise logistic regression analysis was used to determine the presence of container breeding mosquitoes associated

with the explanatory variables. The dependent variables were the presence or absence of *A. aegypti, A. albopictus*, and *C. pipiens* complex in site traps. The independent variables were the thirteen explanatory variables described in Table 1. Mosquito presence was analyzed using a logistic regression (Juliano et al., 2002) model for presence related to trap placement specifically using a binary logit model with a stepwise procedure (SAS Institute 1995, PROC LOGISTIC). Criteria for model inclusion were selected using the stepwise procedure set at the 0.05 level. Further analysis (SAS Institute, PROC GLM) was conducted after the stepwise procedure to determine differences in total mosquito abundance for important factors such as city and sampling period that coincides with Week within the logistic model.

RESULTS

2017 Mosquito Collection: Between May and August 2017, 242 commercial or residential sampling sites were established in six cities along two regional transects in southern Oklahoma (Figure 1). A total of 6,628 female mosquitoes were collected over a total of 210 trap nights/week producing a total of 906 trapping events involving 2118 trap-nights during the summer of 2017 (Table 2). Of the mosquitoes collected, 96% consisted of container breeding species: *A. albopictus* (75%), *Culex pipiens c*omplex (16%), and *A. aegypti* (9%). Of the two types of traps used, GAT traps captured 1,934 (29%) of the mosquitoes collected while BG-sentinel traps captured 4,449 (67%) of the mosquitoes. *Aedes aegpyti* was identified in all cities except Elk City (Table 2). While only one *A. aegypti* collected in Davis and six in Magnum, the majority of *A. aegypti* were collected in Marietta followed by Altus. On the other hand, majority of *A. albopictus* were collected in Mangum and Davis with fewer collected in Marietta and

Altus (Figure 3). The trends in mosquito species abundance tended to increase throughout the summer months with a large increase of numbers after the first of July (Figure 4).

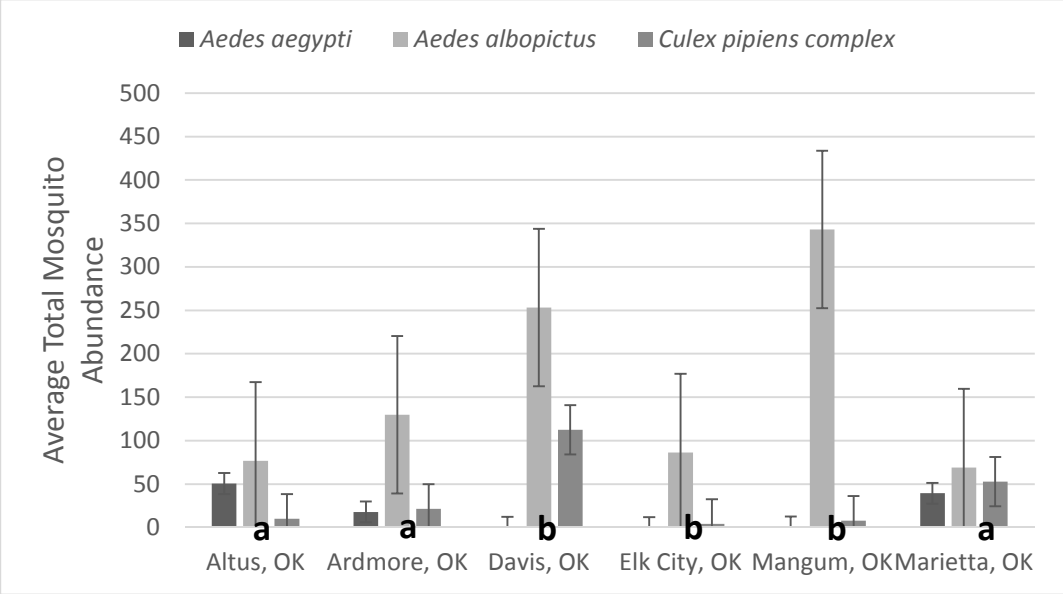


Figure 3: Average total mosquito abundance (*A. aegypti*, *A. albopictus*, *C. pipiens*) per city in southern Oklahoma. *A. aegypti* populations from city to city with the same letter are not significantly different α=0.05.

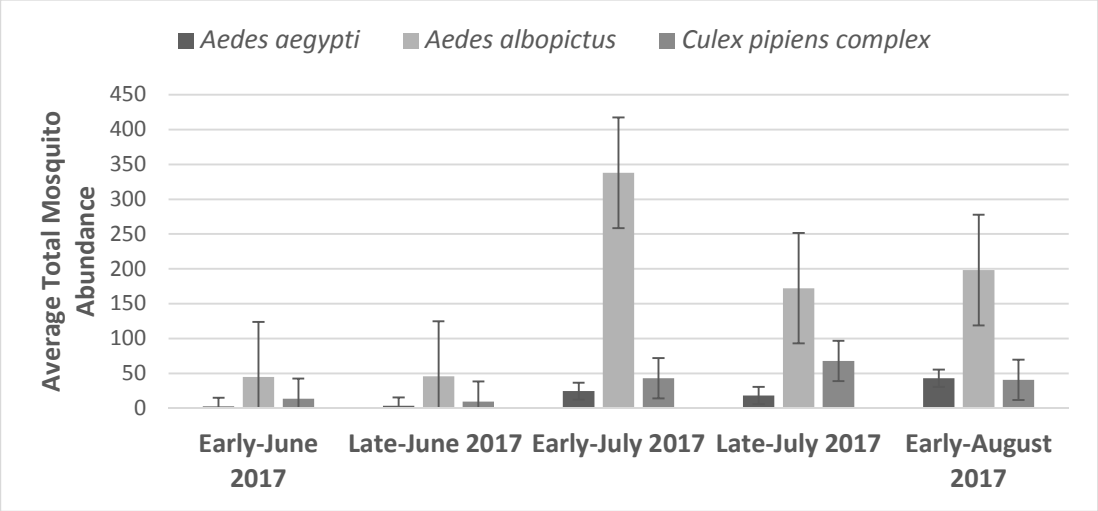


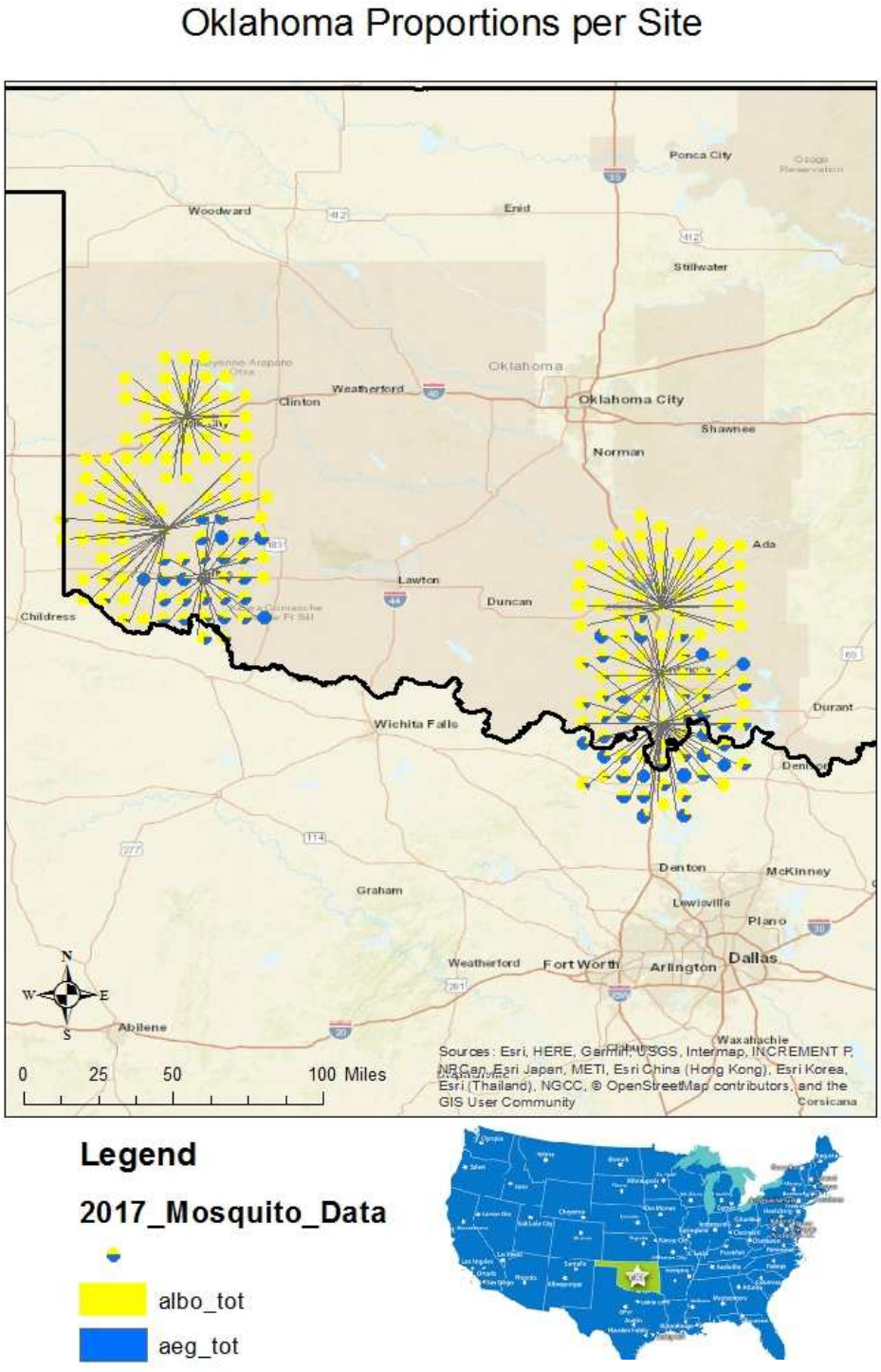
Figure 4. Total abundance of container-breeding mosquito species per round during summer 2017.

Table 2. Mosquitoes collected from 2017 in six Oklahoma Cities using two trapping methods.

Species	City							Trap Type		
	Marietta	Ardmore	Davis	Altus	Mangum	ElkCity	Total	GAT	Sentinel	Total
Aedes aegypti	197	90	1	253	4	0	547	258	289	547
Ae. albopictus	345	649	1266	384	1715	432	4791	1643	3148	4791
Ae. epactius	9	16	35	35	13	11	119	29	90	119
Ae. sollicitans	0	0	0	0	0	3	3	1	2	3
Ae. triseriatus	0	23	25	1	0	6	55	24	31	55
Ae. vexans	0	0	0	1	0	0	1	0	1	1
Anopheles pseudopunctipennis	0	0	0	0	0	1	1	0	1	1
An. puncticpennis	0	0	2	0	2	2	6	1	5	6
An. quadrimaculatus	0	0	0	0	1	1	2	0	2	2
Culex erraticus	2	0	9	1	1	2	15	2	13	15
Cx. nigripalpus	0	0	0	1	0	0	1	0	1	1
Cx. pipiens	264	108	562	50	40	21	1045	33	1012	1045
Cx. tarsalis	0	0	0	0	3	1	4	0	4	4

Cx. territans	0	0	3	0	0	4	7	5	2	7
Psorophora ferox	0	1	0	0	0	0	1	0	1	1
Ps. ciliata	0	0	0	0	0	2	2	0	2	2
Ps. cyanescens	0	0	1	4	1	9	15	11	4	15
Tx. rutilus	3	5	5	0	0	0	13	0	13	13
Total	820	892	1909	730	1782	495	6628	2007	4621	6628

The proportion of *A. aegypti* per city increased the closer the city was to the Red River and Texas border (Figure 5). Interestingly, the proportions of container-breeding mosquito species were similar in the two regional transects. The overall proportion of container species (96% of all mosquitoes collected) in southern Oklahoma were *A. aegypti* (8%), *A. albopictus* (72%), and *C. pipiens* (16%). The overall proportion of *A. aegypti* (9%) to *A. albopictus* (91%) in the western transect was similar to the proportion of *A. aegypti* (11%) to *A. albopictus* (89%) in the central transect. Within the individual cities of the western transect, Elk City had zero *A. aegypti* collected but 100% *A. albopictus,* while Mangum had 2% *A. aegypti* / 98% *A. albopictus* populations. Altus, the most southern city of the western transect produced a 40% *A. aegypti* to 60% *A. albopictus* ratio. Within the individual cities of the central transects, Davis had 0.1% *A. aegypti* vs. 99.9% *A. albopictus*, while Ardmore was 12% *A. aegypti* and 88% *A. albopictus*. The most southern city of central transect, Marietta, produced a 36% *A. aegypti* to 64% *A. albopictus* ratio. Individual city proportion maps are provided in Appendix 5.



bnhspine.com/oklahoma-city-in-usa-map.html

Figure 5: Western and Central Proportion Map of Total *A. aegypti* and *A. albopictus* Collected per Site throughout the Summer of 2017.

***Aedes aegytpi* Confirmation Assay:** Seven unknown mosquito samples from Davis and Mangum were tested by PCR for species identification. Five out of the seven samples (one from Davis and four from Mangum) were confirmed using NCBI Blast with 100% sequence identity with known sequences of *A. aegypti* (KX580042.1; FJ428775.1) while the positive control had 100% sequence identity with a known sequence of Liverpool strain (MF194022.1). Of the two other unknown samples, one had 100% sequence identity for known sequences of *Culex quinquefasciatus* or *pipiens* (GU188856.2; KX709954.1), while there was not enough DNA to determine the identity of the other mosquito.

Canine Heartworm Assay: Of the 670 pools (n=3,298 mosquitoes) of *A. albopictus* tested, six were positive for *D. immitis* based on known sequences (Table 3). Of the five positive *A. albopictus* pools, three were collected in Davis, one in Ardmore, and one in Marietta (Table 3). The majority of *A. albopictus* infected with *D. immitis* were collected in July in urban residential communities in the urban core with volumes of low or medium clutter and half had visible dogs present (Table 4). Of the 165 pools (n=1,026 mosquitoes) of *C. pipiens* tested, none were positive for *D. immitis* DNA. If only one mosquito in each positive pool contained *D. immitis* DNA, the overall prevalence rate in *A. albopictus* for the entire study would be 0.18% while the overall pool infection rate was 0.75%. However, based on the minimum infection rate analysis, *Aedes albopictus* in Davis was the highest (3.97) followed by Marietta (2.88) then Ardmore (1.87) (Table 3). Interestingly, none of the mosquitoes collected in the western transect area were positive for *D. immitis*.

Table 3. Canine heartworm percentage of positive pools and minimum infection rate (MIR) for *Aedes albopictus* collected in six Oklahoma cities between May and August 2017.

City	Total no. mosquitoes	Pool size (range)	No. pools screened	No. positive pools	% positive pools	MIR (Lower/Upper)
Davis	755	1-10	119	3	2.52	3.97 (0.0-8.5)
Ardmore	535	1-10	103	1	0.97	1.87 (0.0-5.5)
Marietta	347	1-10	95	1	1.05	2.88 (0.0-8.52)
Elk City	385	1-10	92	0	0.00	0.00
Mangum	844	1-10	158	0	0.00	0.00
Altus	431	1-10	102	0	0.00	0.00
TOTAL	**3297**		**669**	**5**	**0.75**	

Table 4. Characteristics of *D. immitis*-positive sites where infected *A. albopictus* were collected in Oklahoma, 2017.

City	Date of collection	# mosq in the positive pool	Housing type (Res/Ind)	Location (Core / Rural)	Clutter index	No. containers	# of dogs	Trap type
Davis	June 26-29	10	Res	Core	Med	10	2	GAT
Davis	July 24-27	10	Res	Core	Low	4	0	GAT
Davis	July 24-27	7	Res	Core	Med	26	0	GAT
Davis	July 24-27	2	Ind	Core	Low	0	0	GAT
Ardmore	August 10	8	Ag	Core	Low	0	2	BG-Sentinel
Marietta	July 10-13	1	Res	Core	Med	4	2	GAT

Predictive Parameters for Mosquito Presence

Aedes aegypti

Separate logistic regressions for each mosquito species provided further insight into the different effects that described the surrounding area of trap placement for *A. aegypti* presence (Table 5). The stepwise logistic regression which utilized the explanatory variables associated with trap placement within each individual city and the cities combined as well as the dependent variable of *A. aegypti* presence showed that three cities (Ardmore, OK; Marietta, OK; and Altus, OK) exhibited significant *A. aegypti* presence for criteria selection for the logistic model (Table 5; $\chi^2 = 144.02$ P <0.0001). The combined city effect for *Ae. aegypti* presence reflected that city, week, and traps located in residential areas were the most significant criteria for the logistic model (Table 5; City: $\chi^2 = 144.02$ P <0.0001; Week: $\chi^2 = 77.87$ P <0.0001; Traps located in Residential areas: $\chi^2 = 12.34$ P = 0.0004). The overall model that combined all cities sampled in Oklahoma showed that city was an important factor in determining if *A. aegypti* would be present in traps with a total of 170 unique trapping events with *A. aegypti* present (Table 5). When considering the city effect for *A. aegypti*, further analysis showed that Altus, OK and Marietta, OK had significantly more *A. aegypti* in average total abundance than Davis, OK; Elk City, OK; and Mangum, OK (Fig. 3; df: 5, 29; F = 3.39; P = 0.0186). These two cities are located nearest to the state border with Texas and represent the two cities most likely to have *A. aegypti* present in traps from northward movement from Texas. Week was assumed originally to be significant since mosquito populations typically increase as temperatures increase throughout the summer (Fig. 4 and 7). However, on further analysis, there were no differences in *A. aegypti* abundance from

week to week. However, a numerical trend of increasing abundance is seen from July into August (Fig. 3; df: 4, 29; F = 1.85; P = 0.1506). The predictive variable of traps located in residential areas was important in the overall logistic model that combined all cities for *A. aegypti* presence which is consistent with the ecology of the container-breeding species residing in close contact with human hosts (Table 5, traps located in Residential areas: $\chi^2 = 12.34$ P = 0.0004).

At the individual city level for *A. aegypti*, only three of Oklahoma cities had significant predictive values for *A. aegypti*. The city with the most predictive variables for *A. aegypti* was Altus, OK. Located at the bottom of the western regional transect, predictive variables were week (Table 5; $\chi^2 = 30.10$ P <0.0001), traps located in industrial areas (Table 5; $\chi^2 = 5.35$ P =0.0208), and traps located in areas with no vegetation (Table 5; $\chi^2 = 4.12$ P =0.0424). In the central regional transects, the cities of Ardmore and Marietta had significant predictive variables as well. In Ardmore, predictive variables of week (Table 5; $\chi^2 = 18.27$ P <0.0001) and low vegetation (Table 5; $\chi^2 = 12.15$ P =0.0005) were significant while, in Marietta, the week (Table 5; $\chi^2 = 23.74$ P <0.0001) was the only significant predictive variable.

Aedes albopictus

The combined city effect for *Ae. albopictus* presence reflected that city, industrial areas, city, limited to no vegetation, and dog presence near a trap were the most significant criteria for the logistic model (Table 5; week: $\chi^2 = 68.55$ P <0.0001; traps located in industrial areas: $\chi^2 = 14.15$ P =0.0002; city: $\chi^2 = 22.4$ P =0.0004, 0-10%

vegetation surrounding a trap: χ^2 = 7.89 P = 0.0050; and dog presence: χ^2 = 4.89 P =0.0270). The overall model that combined all cities sampled in Oklahoma showed that week was an important factor in determining if *A. albopictus* would be present in traps with a total of 520 unique trapping events unique trapping events with *A. albopictus* present (Table 5). When considering the city effect for *A. albopictus*, further analysis showed that none of the cities significantly impacted the abundance of *A. albopictus* in average total abundance compared with the other five Oklahoma cities (Fig. 3; df: 5, 29; F = 1.55; P = 0.2111). The city of Mangum, numerically, had a higher total abundance of *A. albopictus* over the other Oklahoma cities while Altus, OK and Marietta, OK had numerically fewer *A. albopictus* on average total abundance than Elk City, OK; Ardmore, OK; Davis, OK; and Mangum, OK (Fig. 3; df: 5, 29; F=1.55; P=0.2111). Week also had no significant differences on the abundance of *A. albopictus* in the six cities (Fig. 3; df: 4, 29; F=2.37; P=0.0797). The lack of significance for city and week is most likely due to the large quantities of *A. albopictus* collected in all of the cities. The predictive variable of traps located in industrial areas was important in the overall logistic model that combined all cities for *A. albopictus* presence which is consistent with the ecology of the container-breeding species residing in close contact with human hosts (Table 5, traps located in Industrial areas: χ^2 = 14.15 P = 0.0002). Areas of limited to no vegetation (χ^2 = 7.89 P = 0.0050) surrounding the traps were significantly influential to *A. albopictus* on a combined city analysis as something that would correspond with the significance of industrial areas. Interestingly, the presence of permanent outside canines (χ^2 = 4.89 P = 0.0270) seems to influence the predictability of *A. albopictus* in a combined city effect, which correlates with the presence of *D. immitis* infected mosquito pools (Table 4).

At the individual city level for *A. albopictus*, all six Oklahoma cities had the most significant predictive values for finding *A. albopictus*. The city with the most predictive variables for *A. albopictus* was Marietta, OK, on the central regional transect with predictive variables of week (Table 5; χ^2 = 15.41 P <0.0001), number of visible containers in the areas (Table 5; χ^2 = 13.69 P =0.0002), tree coverage surrounding a trap in 100 meters (Table 5; χ^2 = 6.47 P =0.0110), and areas with high clutter abundance (Table 5; χ^2 = 7.31 P =0.0069). The other two cities in the central regional transect were both significantly influenced by week, (Ardmore: χ^2 = 8.04 P =0.0046) and (Davis: χ^2 = 15.21 P <0.0001). However, *A. albopictus* in the city of Ardmore was correlated with percent vegetation (Table 5; χ^2 = 4.33 P =0.0374) while in Davis, the amount of urban or impervious surface in a 250meter area surrounding a trap (Table 5; χ^2 = 3.97 P =0.0463) increased the probability of finding *A. albopictus*.

On the western regional transect, the most northern city, Elk City, had four significant predictive values for presence of *A. albopictus* which included 25-50% vegetation around a trap (Table 5; χ^2 = 6.30 P =0.0121), amount of urban is within 100m of a trap (Table 5; χ^2 = 6.12 P =0.0133), week (Table 5; χ^2 = 5.63 P =0.0177), and 10-25% vegetation around a trap (Table 5; χ^2 = 4.96 P =0.0258). The other western cities were both high influenced by the explanatory variable of week, Mangum (Table 5; χ^2 = 26.04 P <0.0001) and Altus (Table 5; χ^2 = 6.45 P =0.0111). The city of Magnum, OK was also influenced by limited vegetation (0-10% coverage) surrounding the trap (Table 5; χ^2 = 11 P =0.0009).

Culex pipiens complex

The combined city effect for *C. pipiens* presence reflected the highest number of significant variables which included week (Table 5; χ^2 = 13.69 P =0.0002); residential (Table 5; χ^2 = 11.06 P =0.0009); city (Table 5; χ^2 = 16.24 P =0.0062); and rural (Table 5; χ^2 = 5.24 P =0.0221). The overall model that combined all cities sampled in Oklahoma showed that week was an important factor in determining if *C. pipiens* would be present in traps with a total of 116 unique trapping events with *C. pipiens* present (Table 5). However, the effect of city on the abundance of *C. pipiens* was not significant as so few were collected in some of the cities (Table 2). However, while not significant, Davis, OK had numerically more *C. pipiens* in average total abundance than other five Oklahoma cities while Ardmore, OK; Altus, OK; Mangum, OK, and Elk City, OK had numerically less *C. pipiens* on average total abundance than Davis and Marietta (Fig. 3; df: 5, 29; F=2.19; P=0.0886). However, when further analysis was conducted, there were no differences in *C. pipiens* abundance from week to week. However, there was a numerical trend of increasing abundance from July into August (Fig. 3; df: 4, 29; F = 0.67; P = 0.6165).

At the individual city level for *C. pipiens.*, most of the Oklahoma cities sampled produced significant predictive values for the presence of *C. pipiens.* On the western regional transect, the most northern city, Elk City, had a single significant predictive parameter of low vegetation (10-25% coverage) surrounding a trap (Table 5; χ^2 = 7.09 P =0.0078) while *C. pipiens* in Magnum were influenced by week (Table 5; χ^2 = 8.86 P =0.0029), which is logical as mosquito population typically rise as temperatures increase

throughout the summer (Fig. 4 & 7). The most southern city of Altus had no significant predictive parameters for *C. pipiens*.

In the central regional transect, the most significant predictive variables for the presence of *C. pipiens* occurred in Marietta where populations were influenced by tree coverage around 100m of a trap (Table 5; χ^2 = 10.72 P =0.0011), 0-10% vegetation surrounding a trap (Table 5; χ^2 = 5.94 P =0.0148), 50-100% vegetation surrounding a trap (Table 5; χ^2 = 10.27 P =0.0014), and tree coverage around 250m of a trap (Table 5; χ^2 = 4.45 P <0.0349). *Culex pipiens* populations in the other two cities in the central regional transect, Ardmore and Davis, were both significantly influenced by different variables. In Ardmore, *C. pipiens* presence was associated with rural areas (Table 5; χ^2 = 6.03 P =0.0141) while, in Davis, they were associated with areas of high clutter (Table 5; χ^2 = 16.22 P <0.0001), residential areas Table 5; χ^2 = 7.06 P =0.0079, and week (Table 5; χ^2 = 6.68 P =0.0097).

Table 5. Logistic Regression Significant Predictive Variables

City	*Ae. aegypti*				*Ae. albopictus*				*Culex pipiens* complex			
	# Trapping Events with Presence	Criteria Included in Model Selection	χ^2	P-value	# Trapping Events with Presence	Criteria Included in Model Selection	χ^2	P-value	# Trapping Events with Presence	Criteria Included in Model Selection	χ^2	P-value
Davis, OK	1	-	-	-	99	*Week*	15.21	<0.0001	30	*High_Clut*	16.22	<0.0001
						Urban_250	3.97	0.0463		*residential*	7.06	0.0079
										Week	6.68	0.0097
Ardmore, OK	34	*Week*	18.27	<0.0001	81	*Week*	8.04	0.0046	19	*Rural*	6.03	0.0141
		Low_Veg	12.15	0.0005		*% Veg*	4.33	0.0374				
Marietta, OK	67	*Week*	23.74	<0.0001	79	*Week*	15.41	<0.0001	18	*Tree_100*	10.72	0.0011
						# containers	13.69	0.0002		*No_veg*	5.94	0.0148
						Tree_100	6.47	0.0110		*High_veg*	10.27	0.0014
						High_clut	7.31	0.0069		*Tree_250*	4.45	0.0349
Altus, OK	64	*Week*	30.1	<0.0001	74	*Week*	6.45	0.0111				
		Industrial	5.35	0.0208								
		No_Veg	4.12	0.0424								
Mangum, OK	4	-	-	-	103	*Week*	26.04	<0.0001	14	*Week*	8.86	0.0029
						No_Veg	11	0.0009				
Elk City, OK	0	-	-	-	84	*Med_Veg*	6.30	0.0121	12	*Low_veg*	7.09	0.0078
						Urban_100	6.12	0.0133				
						Week	5.63	0.0177				
						Low_veg	4.96	0.0258				
Combined OK Cities	170	*City*	144.02	<0.0001	520	*Week*	68.55	<0.0001	116	*Week*	13.69	0.0002
		Week	77.87	<0.0001		*Industrial*	14.15	0.0002		*Residential*	11.06	0.0009
		Residential	12.34	0.0004		*City*	22.4	0.0004		*City*	16.24	0.0062
						No_veg	7.89	0.0050		*Rural*	5.24	0.0221
						Dog Presence	4.89	0.0270				

DISCUSSION

This study provides valuable information regarding the distribution of container-breeding mosquito species in small urban areas in the southern Great Plains. Given the possibility of various arboviruses moving into the state from Texas or by Oklahoma residents travelling to and from counties where outbreaks may occur, the data from this study demonstrate how *A. aegypti* and *A. albopictus* in Oklahoma could be involved in arbovirus outbreaks and provides some predictive factors that might allow for targeted control measures.

Recent studies suggest that Texas, Florida, and California are of particular risk for arbovirus outbreaks transmitted by *Aedes sp*. (Hahn et al., 2017). Potential sites of importance within these states are areas in close association with humans, such as homes, schools or container-rich environments (Hahn et al., 2017). The detection of *A. aegypti* and *A. albopictus* in Oklahoma has created another potential risk of arboviruses in the United States. Since *A. aegypti* resides in urban areas with high abundance of human hosts, cities in close proximity to Texas, such as southern Oklahoma, are at risk for developing arbovirus outbreaks given the wider presence of *A. aegypti* along the Texas border. This study demonstrated that the distribution of *A. aegypti* in southern Oklahoma is highly correlated with the Texas border counties in reference to established *A. aegypti* populations. There was no sign of regional transect effect for *A. aegypti* in Oklahoma. Like similar studies in Florida, the number of *A. aegypti* was greatly reduced as distance increases from highly abundant areas, Texas in the case of this study (Hopperstad and Reiskind, 2016).

While the distribution of *A. aegypti* seems to be concentrated along the southern border of Oklahoma, small numbers were collected in two cities, Davis (n=1) and Mangum (n=4), which are north of where they were previously reported (Bradt, 2017). Interestingly, Mangum is located slightly more north (34.87 ˚N latitude) than sites from a recently published study in the Texas panhandle (34.7˚N latitude) (Peper et al., 2017). As *Aedes* mosquitoes do not travel more than 100-250m to find a host or oviposition site (Reiskind et al., 2016), this study suggests that *A. aegypti* populations may be spreading in a northerly direction via human-aided vehicle-related dispersal either through transport or tires (Soper, 1967; Damal et al., 2013; Metzger et al., 2017). *Aedes aegypti* populations may also be expanding in range due to the changing climate, which may explain why *A. aegypti* was found in a more northward boundary and at a higher latitude than expected in the current study (Metzger et al., 2017). Additionally, the central transect in Oklahoma is divided by the Arbuckle mountains, which are assumed barriers of dispersal (Reisen, 2010) and might restrict the movement of *A. aegypti* into Davis, OK, the northern most city within the central transect. While still unsure whether this collection event on a single mosquito indicates a temporary or established population, it is not obvious how a mosquito species could move from the Ardmore area, below the mountain range, to an urban area above the range without assistance. The potential expansion of *A. aegypti* in a northerly direction increases the risk for an arbovirus epidemic to a greater number of people as the metropolitan area of Oklahoma City, the largest in the state, is not far from Davis (96.6 kilometers). The lack of local mosquito control infrastructure in small towns in Oklahoma, especially for *A. aegypti*, is a limiting factor in controlling or avoiding an arbovirus epidemic in the future. From a public health standpoint, these small urban

clusters may take longer to establish *A. aegypti* populations due to minimal impervious surfaces, reduced vector abundancy, and lower host availability. The ability of *A. aegypti* to proliferate within communities requires an abundance of hosts and suitable habitat for oviposition. While these small urban clusters have host and habitat available, the number is not as plentiful as it might be in a city of 50,000+ individuals. This reduced proliferation time might slow the distribution in an urban 'cluster'; however, it may also play a role in failed detection.

This report of *A. aegypti* in southern Oklahoma is in direct contrast with populations in other states. For example, in a recent statewide larval survey in Mississippi, no *A. aegypti* larvae were collected in 2016, despite a wider ranging surveillance program (Goddard et al., 2017). While the conclusion was that *A. aegypti* was not present, there may have been a sampling issue in that the sampled areas may not have consisted of enough impervious surface that is specific to *A. aegypti*, which should have been carried out closer to the urban core in small cities. Interestingly, Alabama, next to Mississippi, recently reported the discovery of *A. aegypti* in Mobile, an urban area on the Gulf of Mexico (Zohdy et al., 2018). While it is not clear whether it is re-introduction or a product of years of limited to no surveillance in the area, the authors suspect that the species has been re-introduced due to increased urbanization and shipping traffic. In California, *A. aegypti* established quickly through multiple introductions and human-created microenvironments through irrigation and increased vegetation in urban areas (Metzger et al., 2017). Cemeteries or areas of abundant tires have been suggested as good sampling sites for *A. aegypti*; but in Oklahoma, these did not provide any specimens. This is most likely due to the placement of cemeteries outside the towns sampled (Braks

et al., 2003; Vezzani, 2007). In studies where cemeteries are identified as breeding sites for *A. aegypti*, they are more centrally located near the urban core, which is where *A. aegypti* prefer to colonize. The type of facilities management to specific cemeteries or tire repositories in urban areas may play a role in the presence or absence of *A. aegypti* population in the urban areas.

Aedes albopictus

In contrast to *A. aegypti, A. albopictus* was abundantly present in every city sampled, whether in the humid, wetter central transect or the hotter, more arid western transect. The distribution of *A. albopictus* in Oklahoma between 1991 and 2004 in Oklahoma confirmed that *A. albopictus* was already established in many of the counties sampled during this study (Noden et al., 2015a). When considering the established distribution of *A. albopictus*, this study confirms the presence of established populations in two new counties: Jackson county (Altus) and Love county (Marietta). Together with the report of *A. albopictus* in Frederick, Oklahoma (Tillman county) (Bradt, 2017), the total number of counties with established populations is now 72 of 77 counties. As it is assumed that *A. albopictus* is widely distributed throughout all the cities along the southern border of Oklahoma, this would correlate to the confirmed counties in Texas (Hahn et al., 2017) and with more recent reports of *A. albopictus* further north in the panhandle of Texas along the Oklahoma border (Peper et al., 2017). The spread of *A. albopictus* into more northern regions may reflect the effect of climate change on distribution ranges; however, harsh winters may influence this spread (Hahn et al., 2017). In other studies, *A. albopictus* was confirmed to be widely distributed throughout

southern Illinois, Texas, Arizona, California, and Florida (Kim and Stone, 2018; Metzger et al., 2017; Obenauer et al.,2010; Hahn et al., 2017).

Culex pipiens complex L

In the current study, the distribution of *Culex pipiens* complex L. was mainly localized along the central transect. Marietta and Davis had the highest collected populations of *C. pipiens* during July and August in 2017 (Figure 4), which might be due to the higher rainfall that occurred on the central transect during the later summer months (Fig. 6) (Weather Underground, 2018).

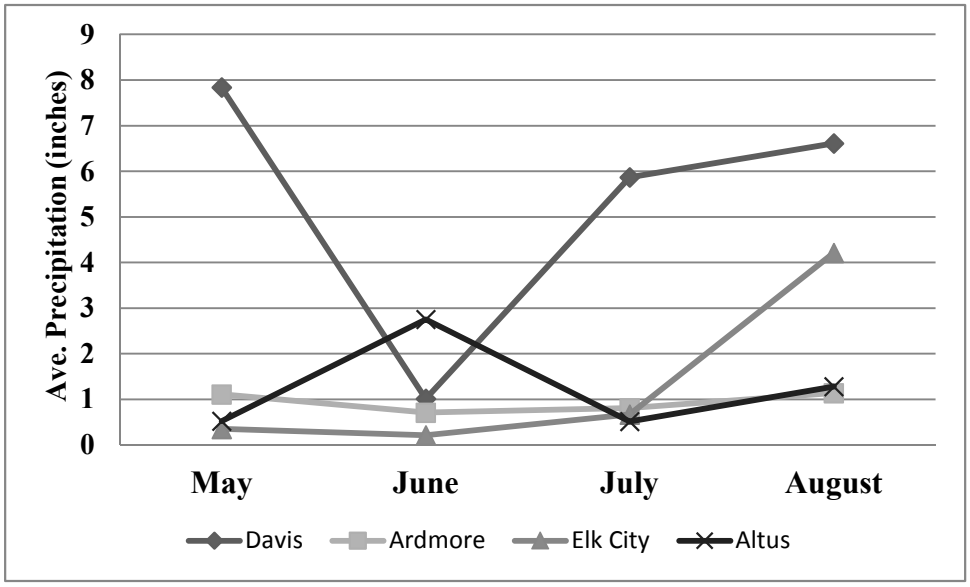


Figure 6. Average Precipitation for Oklahoma cities during the summer 2017 collection period (Weather Underground, 2018).

Surprisingly, more *C. pipiens* were collected using BG-sentinel traps in Davis and Marietta. It was surprising that the use of oviposition water in GAT traps did not attract more females looking for a place to oviposit (Ritchie et al., 2014). While this was possibly due to the use of a commercial lure sold with the trap, a higher diversity of species was collected

using BG sentinel trap as has been reported by others (Johnson et al., 2017). The difference in numbers collected by the two types of traps was also surprising. A New Jersey-based study reported that *C. pipiens* were attracted to *Aedes* gravid traps along with CDC light trap and in rest boxes during a West Nile surveillance project (Williams and Gingrich, 2007). In another study focused on collecting *A. aegypti* using BG-sentinels, around 7% of the mosquitoes collected (n=108) were *C. pipiens*, which demonstrated that traps were used for capturing container-breeding *Aedes sp.* and *Culex sp*. (Krockel et al., 2006). *Culex pipiens* is found in most regions of the United States and is considered to be one of a main vectors of West Nile, which quickly spread across the United States between 1999 and 2003 (Savage et al., 2006; Kilpartick et al., 2008; CDC, 2018). The quick dispersal of a pathogen such as West Nile demonstrates how other mosquito-borne pathogens could spread within Oklahoma and the southern Great Plains if all of the components involved in the nidus of infection are present (Reiskind et al., 2016). Using efficient trapping methods with a variety of traps, surveillance teams can monitor the spread of diseases such as West Nile, different filarial pathogens, and the potential spread of Rift Valley Fever (Mweya et al., 2013; Moise et al., 2018).

Other species

Another interesting component of the study involved other mosquito species that were collected, namely, *Aedes epactius* and *Aedes triseriatus*. *Aedes epactius*, while commonly reported in Oklahoma (Noden et al., 2015b) but not in urban areas (Bradt, 2017), has been associated with containers and coincident with *A. aegypti* larvae in Mexico (Fuentes et al., 2012). An *Aedes* species that establishes in close proximity to humans in rural areas, the vector competence of *A. epactius* is poorly understood

(Farajollahi and Price, 2013). Another species, *A. triseriatus* was not expected to be found in urban cores of the southern cities although it had been found in abundance in Ardmore in previous study (Bradt, 2017). While a common tree hole breeder, *A. triseriatus* may also use containers which can influence the spread of arboviruses such LaCrosse virus and eastern equine encephalitis (Farajollahi and Price., 2013). Another species of surprise was the collection of *Toxorhynchites rutilus septentrionalis* predatory larvae in Marietta on the south central transect. This species was not expected in urbanized areas, however, in Thailand, another species of *Toxorhynchites* was abundant in containers in urban areas, competing with *Aedes* and *Culex* species *(*Weterings et al., 2014). If *T. rutilus septentrionalis* are more present in urban areas of Oklahoma, it may impact populations of *A. aegypti* by lowering the longevity of adults and decreasing their size due to limiting food source and predation threat (Chandrasegaran et al., 2018). Interestingly, a surveillance study of mosquito species found in waste tires in Florida reported similar results with *T. rutilus*, *A. epactius, A. triseriatus, A. albopictus,* and *A. aegypti* (Dinh and Novack, 2018).

PATHOGEN DETECTION

DNA from mature L3 larvae in mosquitoes were detected in six pools from three cities in the central transect that included the cities of Davis, Ardmore, and Marietta. This detection of *D. immitis* only in cities along the central transect was surprising, given the relatively large numbers of *A. albopictus* and *C. pipiens* collected in cities in the southwestern transect. Interestingly, in a national survey that reported *D. immitis* infections in dogs in counties across the United States between 2010 and 2012, the

counties where the cities in the central transect are located had higher infection rates of *D. immitis* infections in dogs than the counties where the cities in western transect are located (Little et al., 2014). In that study, however, positive dogs were only reported in Jackson County where Altus is located, and there was no data from the counties where Mangum and Elk City are located.

Of the container-breeding species tested in this study, *D. immitis* DNA was only found in *A. albopictus.* The presence of dogs in a given area surrounding a trap site was important for infected *A. albopictus*. This is interesting because, given the number of outside dogs counted in the six urban areas during the day as well as some of the socio-economic indicators measured (clutter and containers) in certain communities, we anticipated finding *D. immitis* DNA in at least *A. albopictus* and *C. pipiens* mosquitoes and anticipated having higher mosquito infection rates than what was observed. These anticipated results were due to the results from previous studies from urban areas in Oklahoma that reported *Dirofilaria immitis* in fifteen species of mosquitoes, including *A. albopictus* and *C. pipiens* (Paras et al., 2014; Bradt, 2017). While *A. albopictus* appears to be a significant vector for *D. immitis* in Oklahoma (Paras et al., 2014; Bradt, 2017) and Georgia (Licitra et al., 2010), very few were collected in an urban area in neighboring Arkansas where floodwater mosquitoes (*Aedes vexans*) and *Anopheles quadrimaculatus* were the principal vectors of canine heartworm (McKay et al., 2013). In the Arkansas study, so few *A. albopictus* were collected using UV light traps at dawn and dusk that they were not tested for *D. immitis* DNA (McKay et al., 2013). Given these differences, environmental conditions in specific urban areas may play a large role in whether *A. albopictus* is involved in the transmission of *D. immitis* in a given community.

Although infected L3 *A. albopictus* were previously reported in Oklahoma studies, the prevalence of *D. immitis* in *A. albopictus* was much lower in the current study (0.2%) than in a similar community in northern Oklahoma (1.6%) (Paras et al., 2014). This could possibly have been due to a variety of reasons including types of traps used (GAT/Sentinel vs Light traps with dry ice), placement of traps, and treatment regimens for anti-helminthic drugs used by the residents in the homes where sampling occurred. As the number of positive samples was similar to another study conducted in summer 2016 throughout Oklahoma (Bradt, 2017), there is a possibility that something may have occurred in the process of DNA extraction that inhibited the PCR reaction. As is common to PCR reactions (Schrader et al., 2012), the use of crudely extracted DNA, which works for tick and flea pathogen detection (Noden et al., 2017; Noden et al., 2018), may not be an appropriate way to extract DNA from mosquitoes for an accurate detection of *D. immitis* as other studies have utilized more expensive extraction kits in their methodologies (McKay et al., 2013; Paras et al., 2014). At the time of writing, the author is in the process of using dilutions of the pooled samples to improve the detection sensitivity of the reaction. As was mentioned above, another difference might be the use of a Heartworm preventive medication in urban communities that might have an effect on detection in infected mosquitoes in the southern Oklahoma. We are not aware of any differences but advocacy by veterinarians and most animal shelters in local communities is well established and may be making a difference in canine infections in the communities sampled.

The identification of *A. albopictus* as the main vector of canine heartworm in this study is not only important from a community perspective, it is also indicative of a more

common threat posed by the species. As a day feeder, *A. albopictus* is in communities throughout the day, providing a higher risk for the spread of pathogens like *D. immitis* to domestic and wild animals in rural and urban communities (Licitra et al., 2010). This risk of spreading *D. immitis* is small when compared with the risk the species poses for human arboviruses such as Dengue and Chikungunya should they enter the region via an infected traveler (Hahn et al., 2017). The focus on *A. albopictus* as a heartworm vector in this study only provides a basis on which to demonstrate its potential to spread even more deadly pathogens within a short period. To assist with this process, these cities, where the species has been collected, have been alerted to this danger in summary reports provide to each of the OSU extension agents within the respective counties (Appendix 6).

MOSQUITO PRESENCE DATA

Predictive modeling is often associated with temperature, relative humidity, and landscapes for container-breeding mosquitoes across the United States and other parts of the world (Estallo et al., 2008; Murdock et al., 2017). In the current study, biotic factors that may influence container-breeding mosquito populations were measured and analyzed from six urban sites in southern Oklahoma. Southern Oklahoma consists of small urban cluster cities of relatively small populations of around 2500+ people (Bureau, U., 2018). These small urban clusters are called 'urban' because impervious surface is present but may still be considered 'rural' due to size of human populations and abundance of vegetation throughout the communities. This landscape of semi-urban and semi-rural may influence the distribution of mosquito species, namely container-breeding species, throughout individual cities as well as at the regional level. The primary mosquito of interest in this study was *A. aegypti* due to its superior ability to transmit a variety of

human diseases around the globe and the recent finding of the species in the northern Texas/Southern Oklahoma region after being not detected for over 70 years (Bradt, 2017; Peper et al., 2017; Hahn et al., 2017). On the wider scale for predictability, Oklahoma's *A. aegypti* populations were localized in specific cities in the southern portion of the state, followed by the week in which sampling occurred. This is logical because as temperatures increased throughout the summer months, *A. aegypti* presence typically increased as well (Figures 4 and 7 (Weather Underground, 2018) as has been also

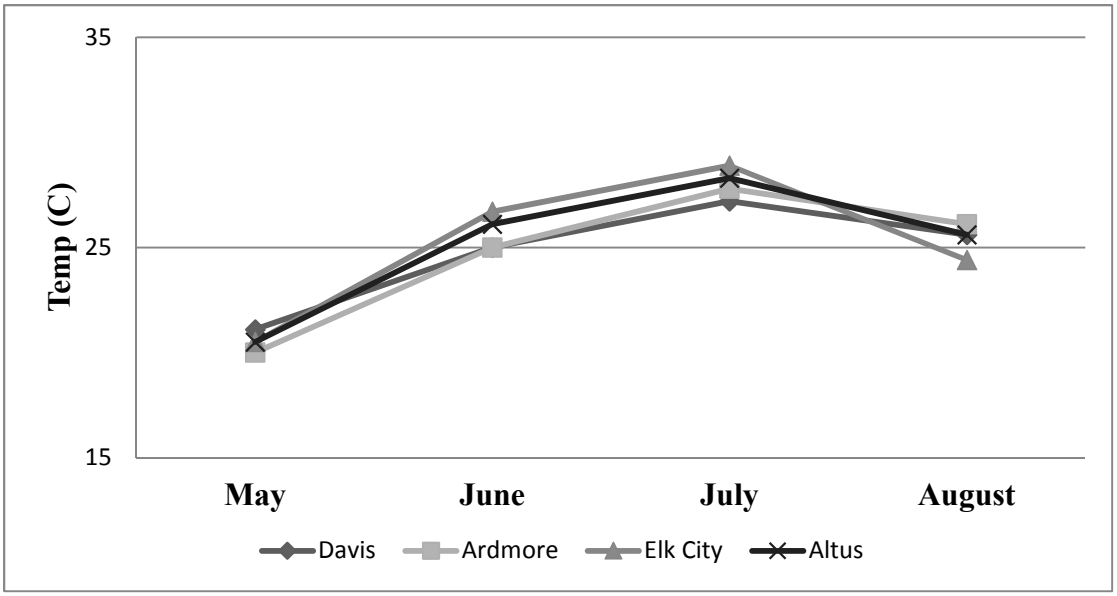


Figure 7. Oklahoma Average Temperature for the months of summer 2017 collection period. (Weather Underground, 2018)

documented in south Texas (Srinivasan et al., 2017). The predictive significance of finding *A. aegypti* in residential areas is consistent with other studies focused on the ecology of the container-breeding species due to their documented contact with human hosts and container-breeding habitat (Braks et al., 2003; Hopperstad and Reiskind, 2014).

At the individual city level for *A. aegypti*, predictable variables varied between cities, most likely due to specific characteristics of the landscape and structure of the

cities themselves. All three cities that produced predictive variables for *A. aegypti* were influenced most significantly by week of sampling, reinforcing again the importance of time of year and precipitation cycles for trapping of established population in Oklahoma, possibly the whole of southern Great Plains region. Another variable was the amount of vegetation surrounding the trap sites, providing cover or obstructing odors from traps from females looking to oviposit or to rest (Johnson et al., 2017). Interestingly, 10-25% vegetation surrounding a trap was predictive for *A. aegypti* in Ardmore while 0-10% vegetation surrounding a trap was predictive in Marietta. While there is some difference between the two predictive categories, this suggests that low vegetation, in general, in areas surrounding traps may enhance the collection of *A. aegypti* in central Oklahoma. These areas of low vegetation also correlate with an increase in impervious surface in urban areas which is known to be predictive as the primary habitat of *A. aegypti* in other areas (Braks et al., 2003: Tsuda et al., 2006; Hopperstad and Reiskind, 2016;). In summary, *Aedes aegypti* in Oklahoma appears to be influenced by the time of year, while other factors may influence the presence at the level of an individual city.

The ecology of *A. aegypti* may be different than other areas of the US due to establishing within cities with limited impervious surface and rural environments close to town (Hopperstad and Reiskind, 2016). Urbanization usually alters the ecology of vector systems by the increasing human population in a single area which is the primary food source for peri-domesticated mosquitoes such as *A. aegypti* and *A. albopictus* (Paras et al., 2014). While most of the parameters were consistent with other studies in the United States, small differences among ecoregions in Oklahoma may play a role in influencing the monitoring, control and management of *A. aegypti* in small urban areas. When

considering the city effect for *A. aegypti*, further analysis showed that Altus, OK and Marietta, OK had significantly more *A. aegypti* in average total abundance than Davis, OK; Elk City, Ok; and Mangum, OK. These two cities are located nearest to the state border with Texas and represent the two cities most likely to have *A. aegypti* present in traps from northward movement from Texas. The average total *A. aegypti* abundance in Ardmore, OK was not significantly different from the two cities with the highest numbers of collected *A. aegypti* (Altus, OK and Marietta, OK) or the other cities with either no or low abundance of *A. aegypti*, which possibly represent a transitional zone of where this mosquito is expanding northward.

The invasion of *A. albopictus* throughout the US is characterized by establishment in rural and urban areas (Li et al., 2014; Murdock et al., 2017). The numerous predictive variables for *A. albopictus* in Oklahoma generated in this study displayed a wide, non-specific distribution. Similar to *A. aegypti*, the main predictive variable for this dominate species across the whole region is week of sampling, linking again to increases in temperatures throughout the summer months (Figure 7). However, unlike *A. aegypti, Aedes albopictus* was found in all cities sampled and not only established in cities in southern parts of Oklahoma. During the summer of 2017, *A. albopictus* was more likely to be collected in industrial areas on a combined city effect, which is also different than *A. aegypti* relationship with residential areas. While *A. albopictus* was collected in all cities, the majority were collected in a single trapping week in two cities which significantly influenced the effect of city that was observed. While it is not clear why these two major trapping events occurred for this species, it does create some potential bias in the data that needs to be considered. Other significant predictive variables

included areas of no vegetation (0-10%) and the presence of dogs at the trapping site. Finding *A. albopictus* associated with areas with limited vegetation surrounding the trap is interesting because others have reported *A. albopictus* to be associated with vegetation (Obenauer et al., 2010). The presence of dogs may also indicate that *A. albopictus* may be establishing in areas with alternative hosts to humans. While not possible to know whether this might be true, due to not testing for host preference, the possibility that *A. albopictus* in Oklahoma is associated with industrialized areas with low vegetation and the presence of dogs may indicate that there may be some competitive interactions going on with other species that are changing the behaviors of the species.

At the individual city level, as was found with *A. aegypti*, the week in which sampling occurred significantly influenced the collections of *A. albopictus* in all cities. However, the other predictive variables among cities were dissimilar. In the central transect, *A. albopictus* populations in Davis were influenced by the percent of impervious surface surrounding a trap site while populations in the southern cities were impacted by varying percentages of vegetation and tree canopy coverage. Some of this difference might be due to the high rainfall found in Davis compared with the other areas (Figure 6). So much rainfall in one area might reduce the importance of some of the measured variables. The influence of number of containers and high clutter on the presence of *A. albopictus* in Marietta was also interesting. The significance of clutter correlates with the significance of number of containers in the areas around the trap site based on a Google image analysis, meaning typically both front and back sides of home, which would have increased the number of containers providing breeding sites of *A. albopictus*. While in areas such as Davis, OK and Elk City, OK, impervious surface around a trap was

significant, but in cluttered Marietta, OK, tree coverage around a trap was significant. The habitat preferences of *A. albopictus* are vast and may cause challenges in controlling the species during an arbovirus outbreak.

Lastly, this study provided some predictive variables associated with *C. pipiens* populations, a container-breeding species with a broader feeding preference and generalized habitat preference (Farajollahi et al., 2011). Like the other container breeding species in Oklahoma, collections of *C. pipiens* were significantly influenced by week of sampling, again probably involving increasing temperatures and precipitation events (Fig. 6 and 7). This is consistent with a study in New Orleans that reported that *C. pipiens* abundance was highly influenced by temperature during the week (Moise et al., 2018). *Culex pipiens* was also found in the central proportion of the state in numerically higher abundance than in the western portion of the state (Fig. 3). However, it was interesting to note that on the scale of the combined six cities, residential and rural areas were most likely to be environments where *C. pipiens* could be collected. While at the individual level, *C. pipiens* was associated with residential areas in Davis, OK, which aligns with others (Farajollahi et al., 2011; Paras et al., 2014). At the individual level, *C. pipiens* was mostly influenced by rural areas (those more vegetative areas on the outskirts of urban areas), the amount of vegetation surrounding trapping sites, and tree canopy coverage, both at 100 meters and 250 meters, around the trapping site. These predictive variables correlate with another study which reported that *C. pipiens* is highly associated with urbanized areas (Savage et al., 2006; Moise et al., 2018). Unlike the other cities, *C. pipiens* presence in Davis, the northern most city on the central transect, was most influenced by high clutter in residential areas. The presence of high clutter, together with

the high amount of precipitation observed, may concentrate the species in a given residential area which would provide an increased number of hosts (Savage et al., 2006; Moise et al., 2018). *Culex pipiens* in Oklahoma, although often associated with both rural and urban communities (Paras et al., 2014), may be influenced directly by the small size of urban clusters and host availability.

Limitation of Study

While all attempts were made to adjust for limitations, it is difficult to plan for everything in a study as wide in geography and labor-intensive as this one. A number of components during the mosquito collection may have contributed to better predictive parameters. For example, restriction of collection to the front lawns or businesses may have limited the actual numbers of mosquitoes collected as well as influenced the prediction variables of number of containers counted and clutter as estimates could only be made from what was obvious and Google Earth images which may not be up-to-date. Another factor was the use of GAT traps instead of CDC Light traps with dry ice as a CO_2 source as is common in such studies (Bradt, 2017; Reiskind et al., 2016). GAT traps were used predominately to reduce the cost of such rigorous sampling scheme and were successful in mainly collecting *Aedes* species as was planned. The addition of BG-Sentinel traps around the outer areas of the cities may have caused a bias in numbers of *A. albopictus* due to *A. aegypti* being more centrally located and confined to the areas where GAT traps were used (Paras et al., 2014). While two years of sampling would have increased the predictive power of the study, another limitation was the required stopping of the study in the middle of August 2017. The termination of sampling was a result of funding issues that caused researchers to be unable to support the rigorous trapping

scheming. Populations of *A. aegypti* and *A. albopictus* normally begin to increase in Oklahoma in August and September (Bradt, 2017) so by not sampling throughout the whole mosquito season, certain biases may have been introduced into the study results. The ability to extend this collection period further into the summer would have provided a better representation of *A. aegypti* during their peak season. Finally, as with any similar study with many parameters to manage, it may have been helpful to have a better understanding of how geographical information systems work prior to starting in order to achieve a more complete picture of what went on between parameters and develop 'hot spot' maps for each species in the urban areas sampled.

CHAPTER IV

CONCLUSIONS

In conclusion, local health authorities can make informed management protocols for potential arbovirus outbreaks by better understanding the ecology of container-breeding mosquito species in Oklahoma. While Oklahoma does not currently have any arboviruses transmitted by *Aedes* species, this study provides some data regarding what could happen should an outbreak occur, especially in the southern portion of Oklahoma. As such, local officials may need to be aware of city specific differences that are involved with these three mosquito species. Not only do these species have different feeding behaviors, but also have different habitat preferences in different urban clusters and regions of Oklahoma.

While the statistical regression results for combined cities may be useful in large urban communities, such as Oklahoma City, smaller urban communities, like the ones used for this study, are quite diverse. The landscapes of these smaller urban clusters vary in size and neighborhood/business plot structures plans that might cause a differential effect for each mosquito species within individual cities.

. Officials also need to be concerned about the amount of clutter within residential areas providing habitat for *Aedes* species, while more vegetation around these cluttered areas will provide resting sites for *Culex* species as well. Additionally, city officials need to be mindful that *A. albopictus* appear to prefer industrial areas while *A. aegypti* and *C. pipiens* appear to prefer residential areas.

As such, management of these species is quite different and should be addressed as control protocols are implemented. In the last 10 years, Oklahoma communities have developed mosquito control programs focused on reducing the risk of West Nile virus and *Culex* mosquitoes. However, given the potential risk for arboviruses by urban breeding *Aedes* species, officials should also focus on community-wide cleanup of containers and clutter instead of just relying on early dawn and late dusk insecticides sprays used for *Culex* sp. Conversely, since *Aedes* feed primarily through the day, local health authorities should stress the importance of potential risk in the mid-summer months when container species population and abundance are prevalent in southern Oklahoma. The use of personal protection such as long clothing and insecticide sprays such as DEET are effective safety precautions against mosquito borne disease when participating in outdoor activity. In prevention of reducing canine heartworm in southern Oklahoma, control of mosquito vectors may reduce the risk of infection but only with the concurrent use of heartworm preventive from a local veterinarian.

While the information gathered from this study was informative for beginning to develop effective mosquito control management strategies, more research specifically into the ecology and distribution of *A. aegypti* would be helpful. The possible expansion or dispersal northward of this important vector species in Oklahoma may cause even

more challenges for control efforts if the mosquito species manages to establish in large urban communities such as Oklahoma City. By continually monitoring *A. aegypti* distribution in the state, it might be possible to envision whether potential outbreaks of arbovirus may occur in the years to come.

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APPENDICES 1

Summer 2017 Information Brochure

Aedes aegypti: The Yellow Fever Mosquito

Master's Research Project

I plan to help protect local communities from disease outbreaks spread by mosquito populations in Oklahoma. My research objectives include:

- Determining *Aedes aegypti* and *Aedes albopictus* distribution in exurban areas in central and western Oklahoma
- Identifying prevalence of *Dirofilaria* and *Plasmodium sp.* In container breeding mosquito species collected in exurban areas of Oklahoma.
- Predicting *Aedes sp.* distribution in exurban areas using habitat modeling.

Content Information

Things To Remember!

- Why mosquito research is important to public health
- How diseases are transmitted
- How to protect yourself (DEET)
- Mosquito Prevention Practices

For more information or questions

Please contact:
County Extension Offices:

Ardmore 580-223-6570
Davis 580-622-3016
Marietta 580-276-3385
Altus 580-482-0823
Elk City/Sayre 580-928-2139
Mangum 580-782-5503

Oklahoma State Department of Health

STATE EXTENSION

SURVEILLANCE OF CONTAINER BREEDING MOSQUITOES
127 Noble Research
Oklahoma State Univ.
Stillwater, OK 74078

Tri-fold Back

Yolonia, FL-DOH

SURVEILLANCE OF CONTAINER BREEDING MOSQUITOES

Oklahoma Department of Health And Oklahoma State Department of Entomology and Plant Pathology

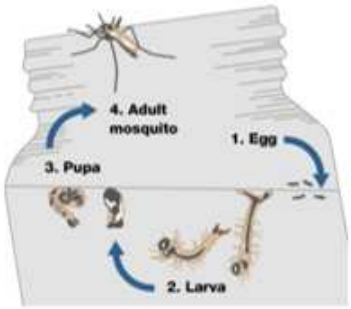
Tri-fold Brochure Front

Disease Transmission

Accidently, mosquitoes often transmit pathogens during blood feeding. During saliva injection, the mosquito can transmit viruses, worms, and protozoans into the hosts' body. Possible pathogens transmitted by *Aedes aegypti* consist of

- Zika Virus
- West Nile Virus
- Malaria
- Dengue Fever
- Chikungunya
- Dog Heart Worm

Content Information



Mosquito Life Cycle

One Week Development=

1 female/ 100-200 mosquitoes

- Egg submerged in water can hatch within 24 hours
- Larvae reside and feed in water container for 7 days before pupation
- Adults emerge following pupation and live 3-4 weeks

Content Information

Management Practices

Prevent outbreak of mosquito populations. By controlling mosquitoes, citizens can reduce the spread of infectious diseases. Keep your family safe by these prevention practices.

- Dump water containers weekly
- Remove waste containers
- Place lids or small netting on remaining containers
- Wear protective long clothing
- Use insect repellants containing DEET as directed on label
- Remain indoors when mosquitoes are most active: early morning and late afternoon

Content Information

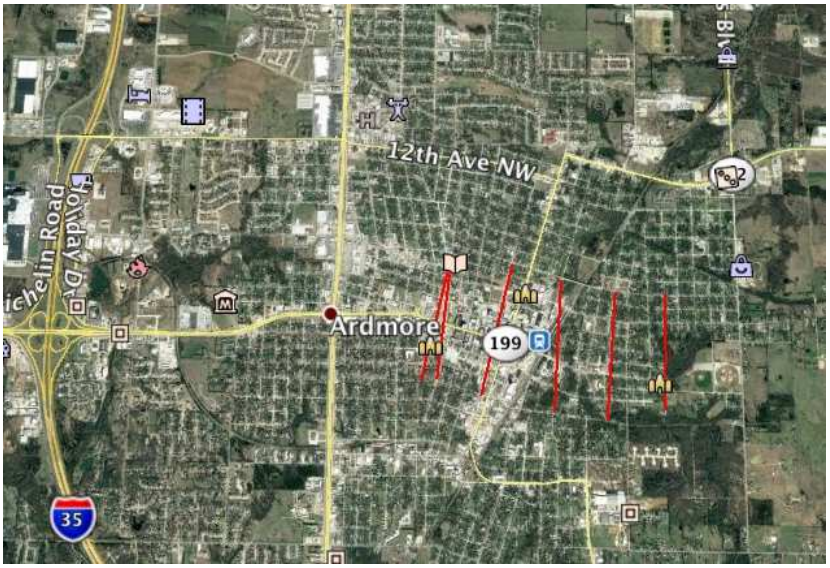
Appendix 2

City Transects

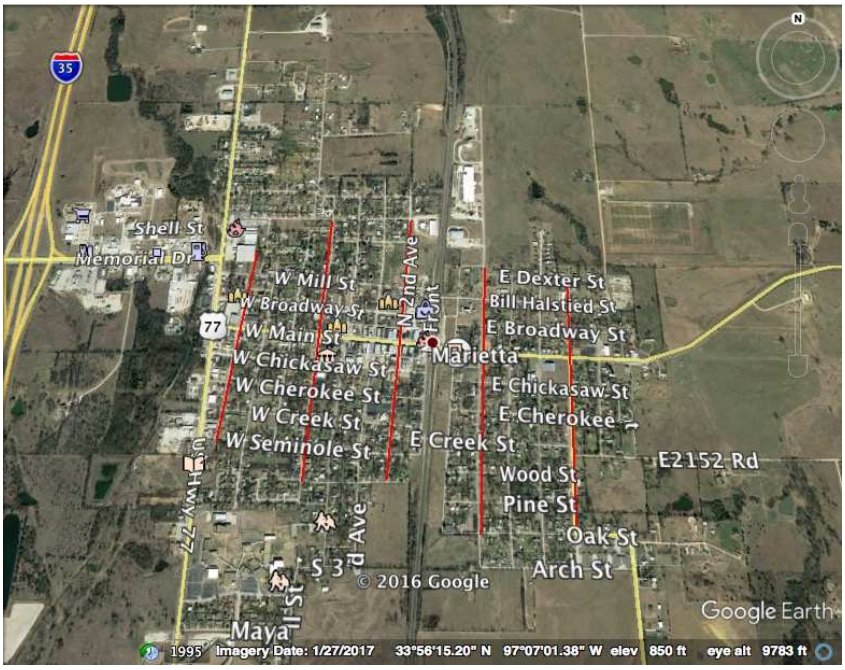
Central Region

1. City of Davis

2. City of Ardmore

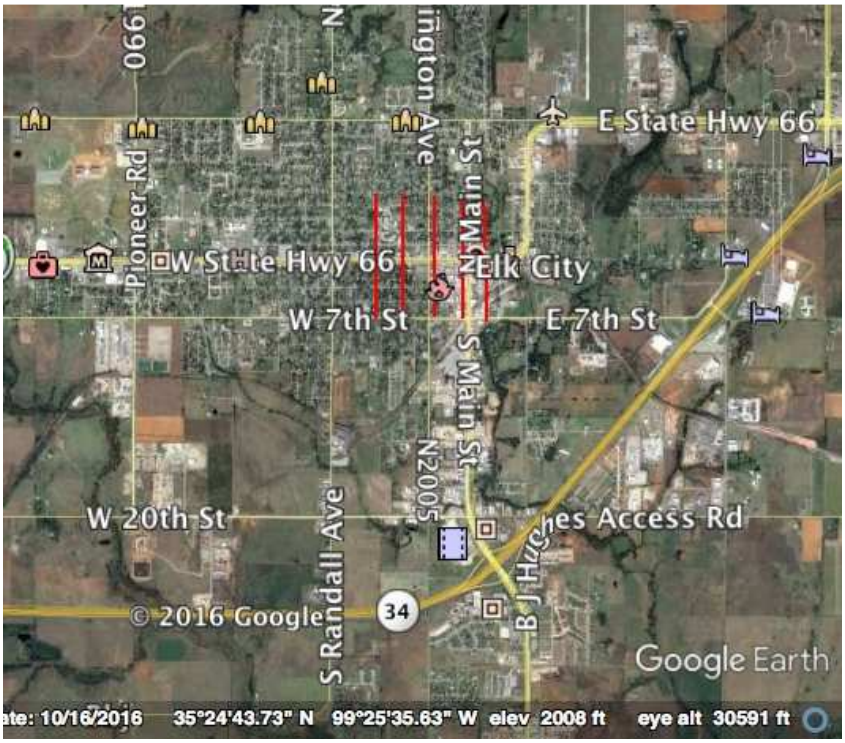


3. City of Marietta



Western Region

4. City of Elk City



5. City of Mangum

6. City of Altus

Appendix 3

Aedes Gravid Trap Sites in Cities

Central Sites

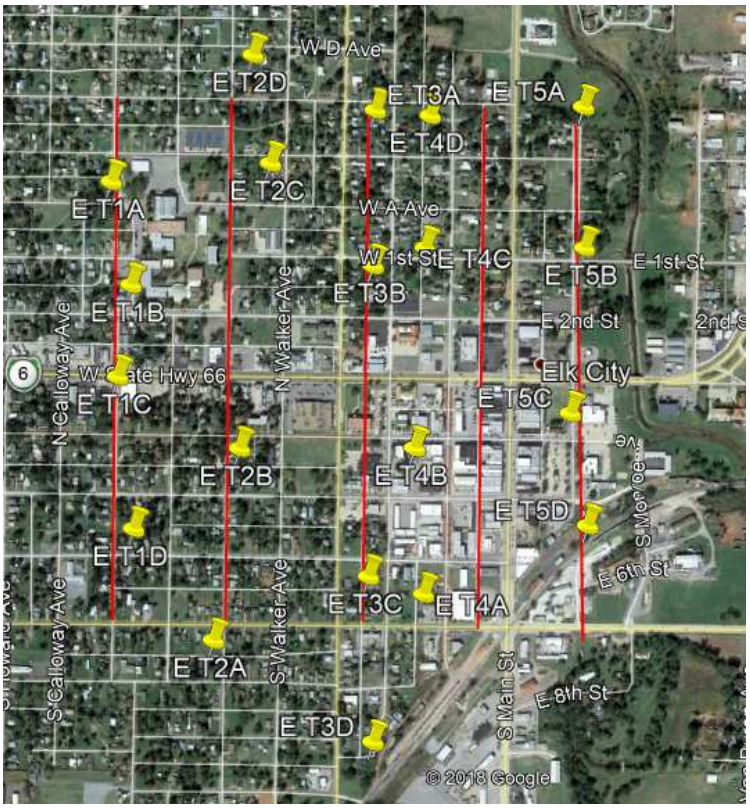
1. **City of Davis**

2. **City of Ardmore**

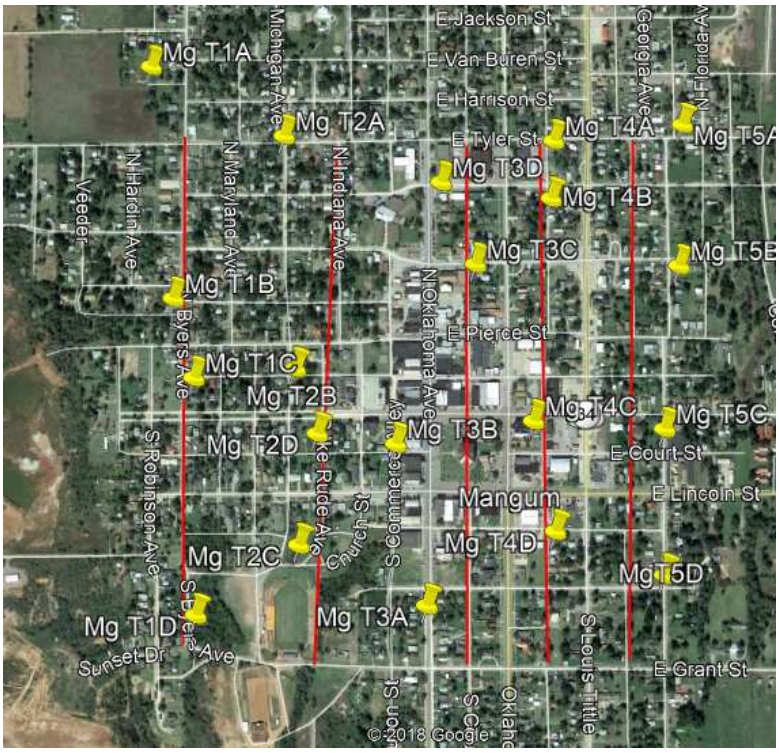
3. City of Marietta

Western Sites

4. City of Elk City



5. City of Mangum



6. City of Altus

Appendix 4

BG-sentinel Trap Sites in Cities

Central Sites

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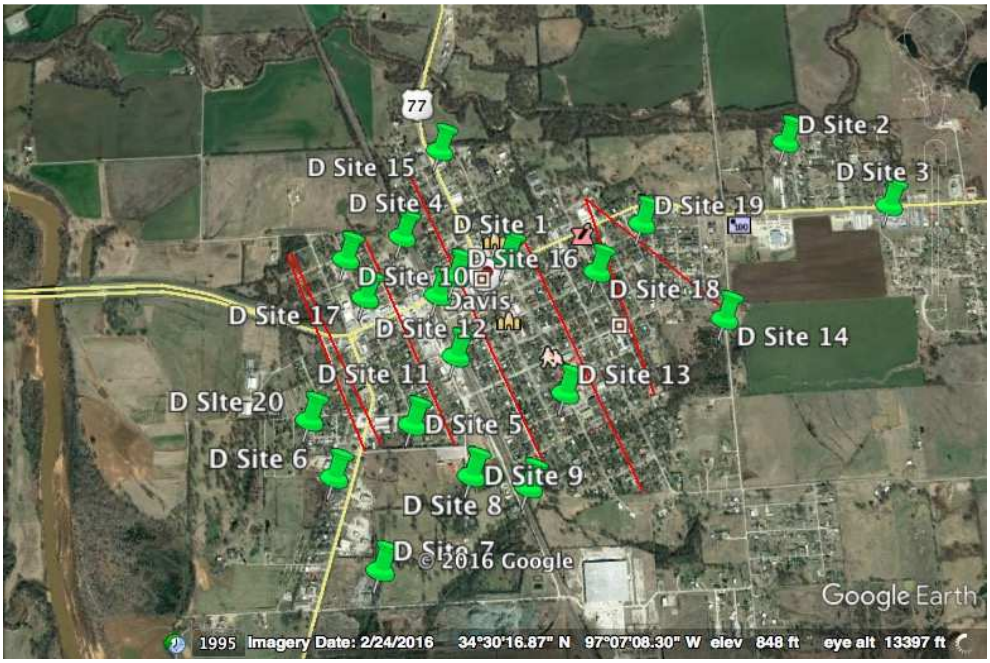
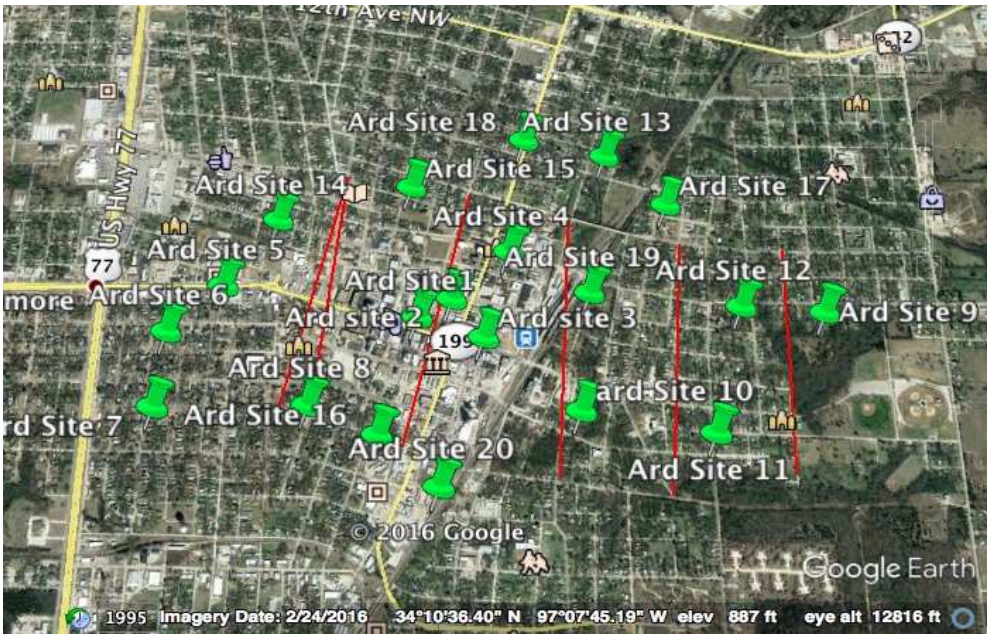
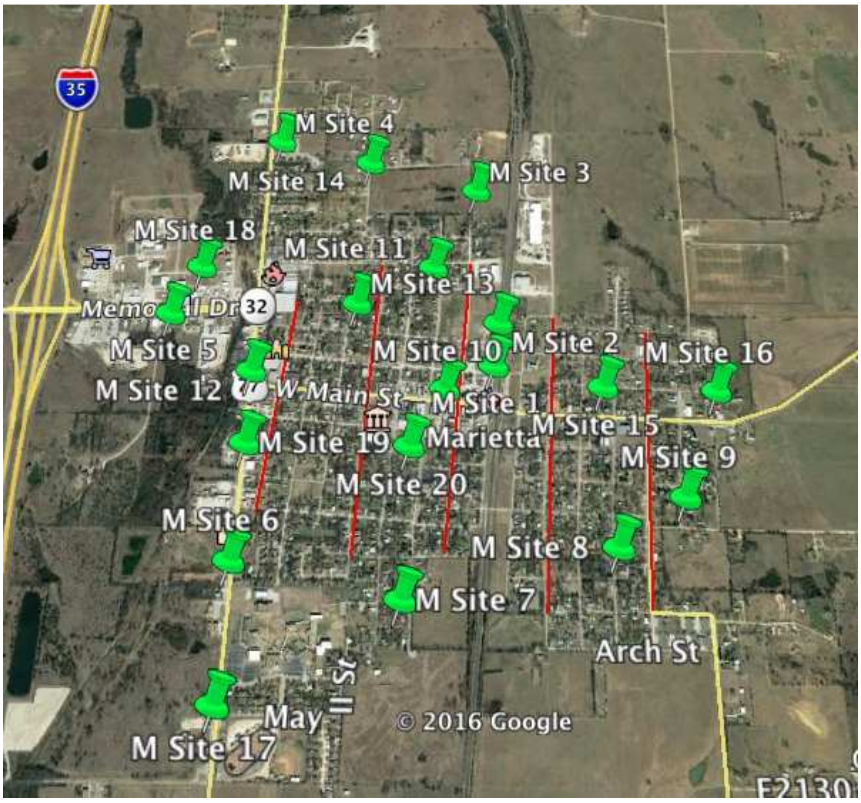


2. **City of Ardmore**

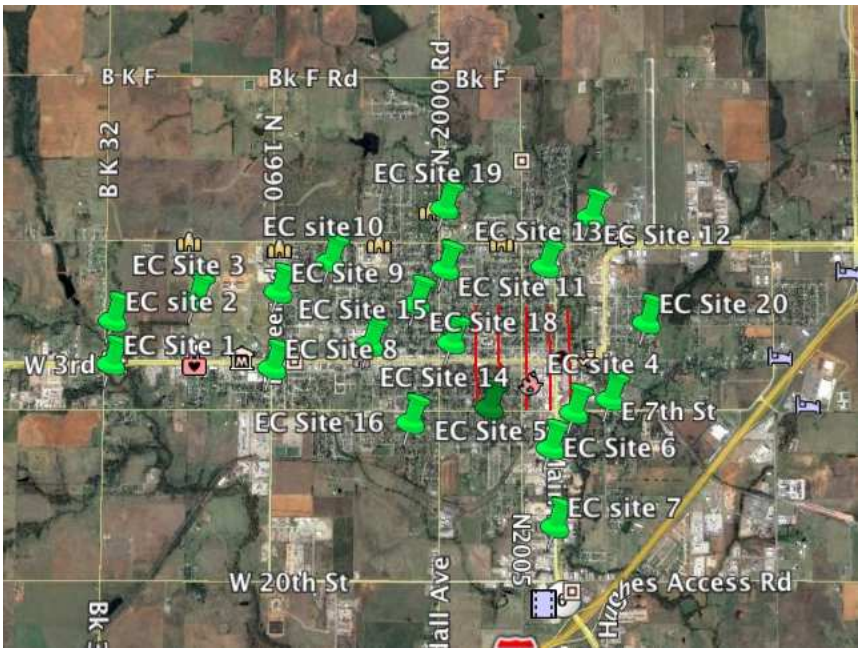


3. City of Marietta

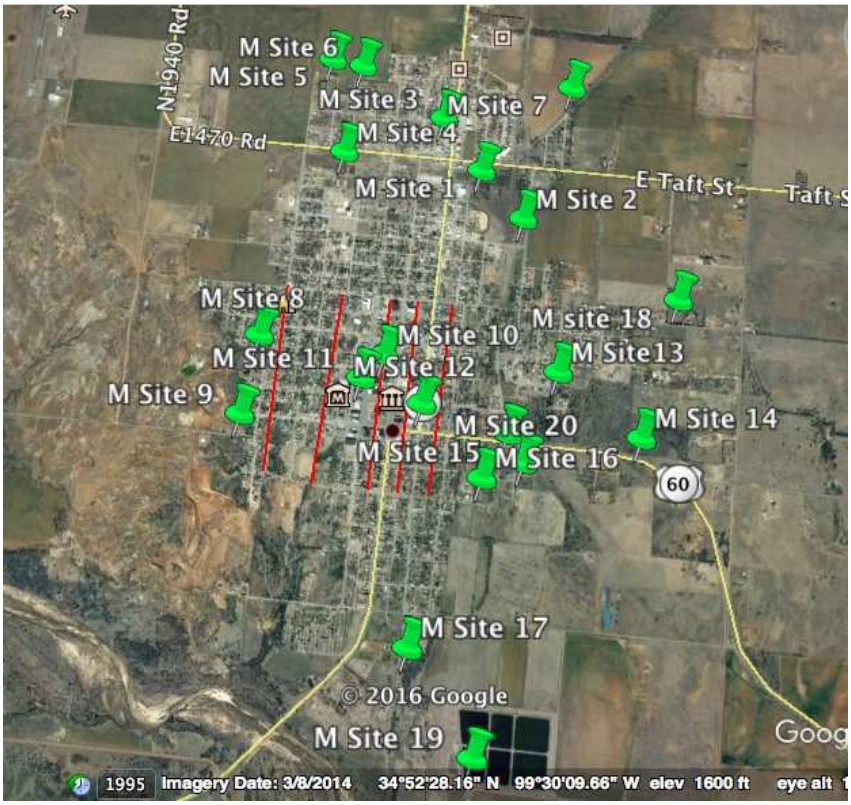


Western Sites

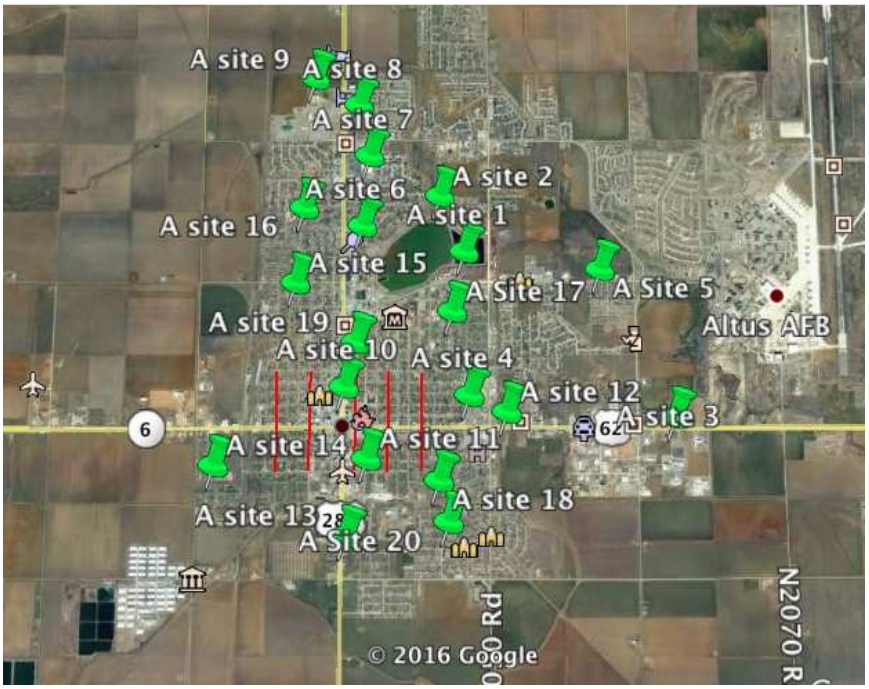
4. City of Elk City



5. City of Mangum



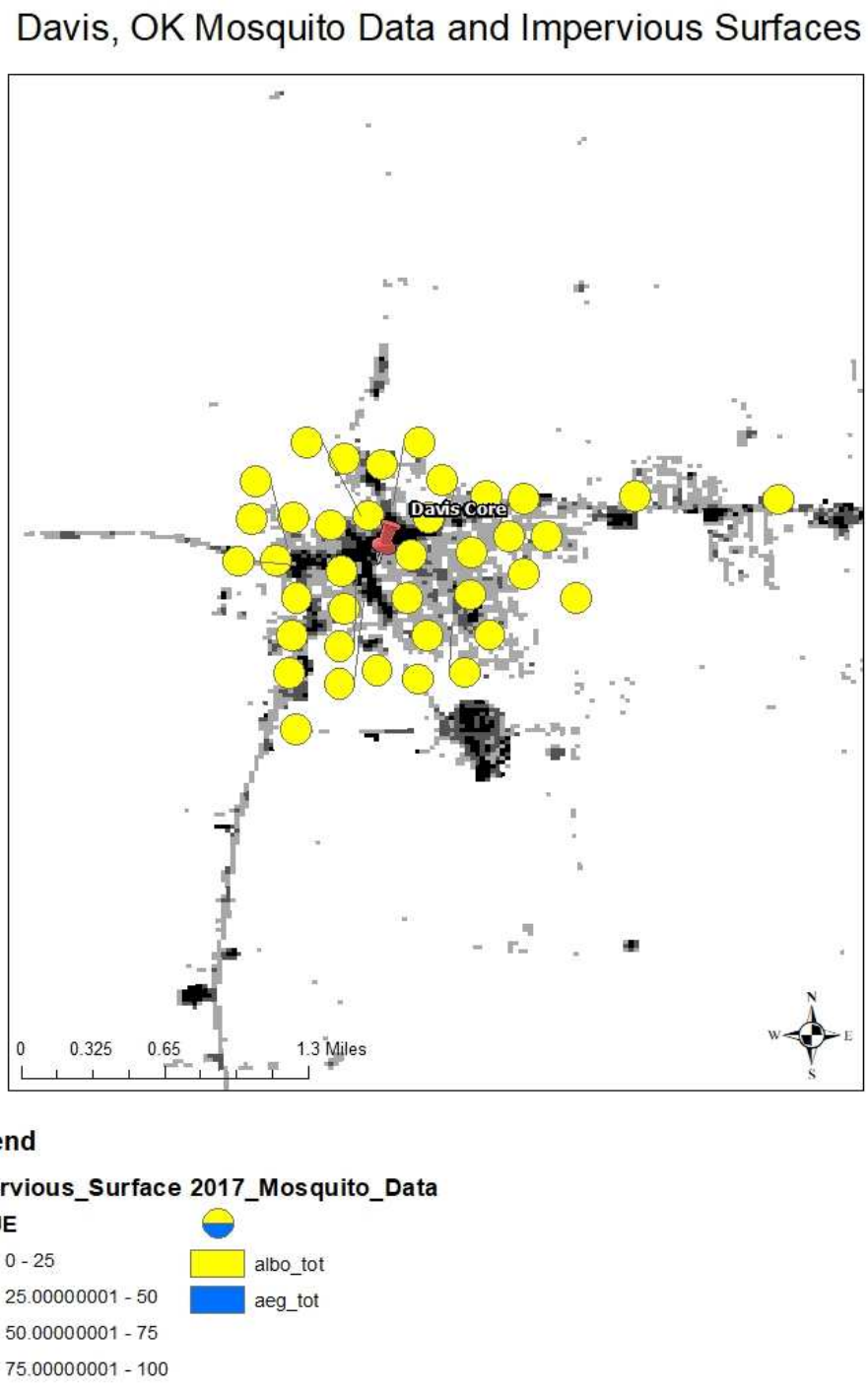
6. City of Altus



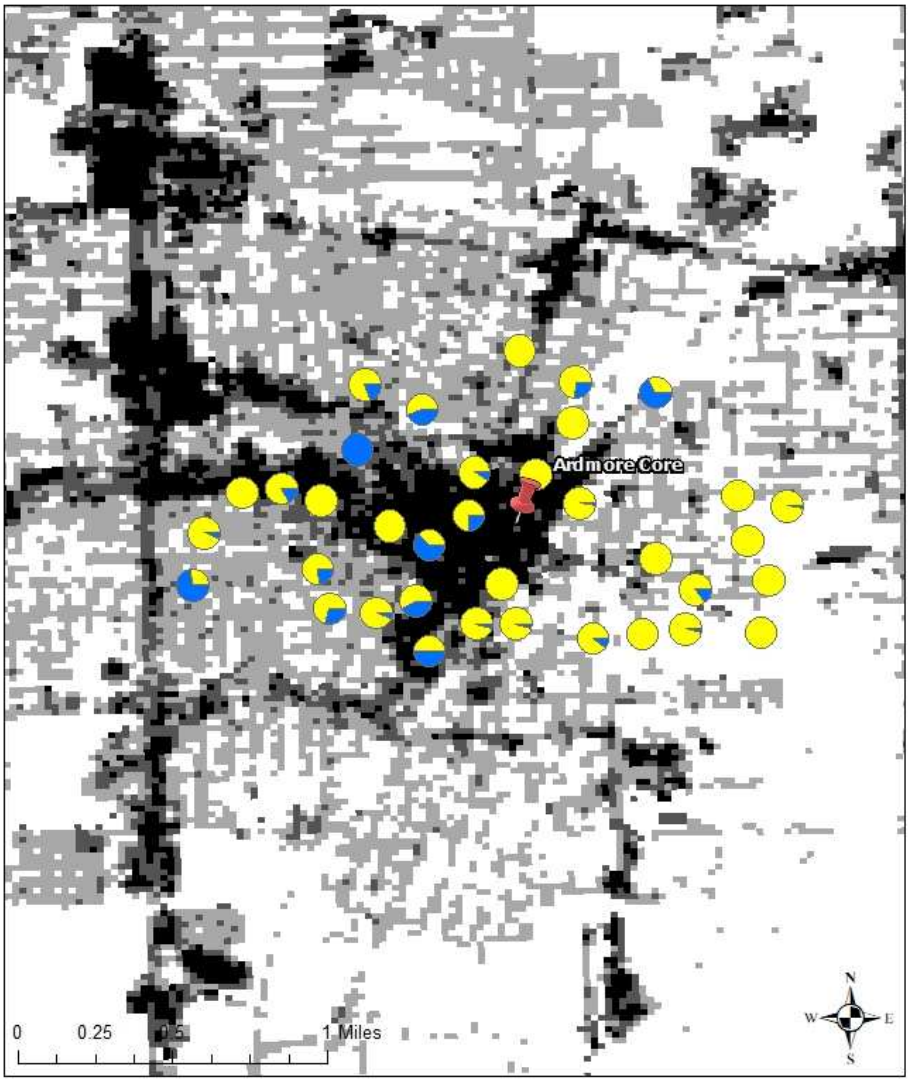
Appendix 5

Proportions by City

Central Region Proportion Maps



Ardmore, OK Mosquito Data and Impervious Surfaces



Legend

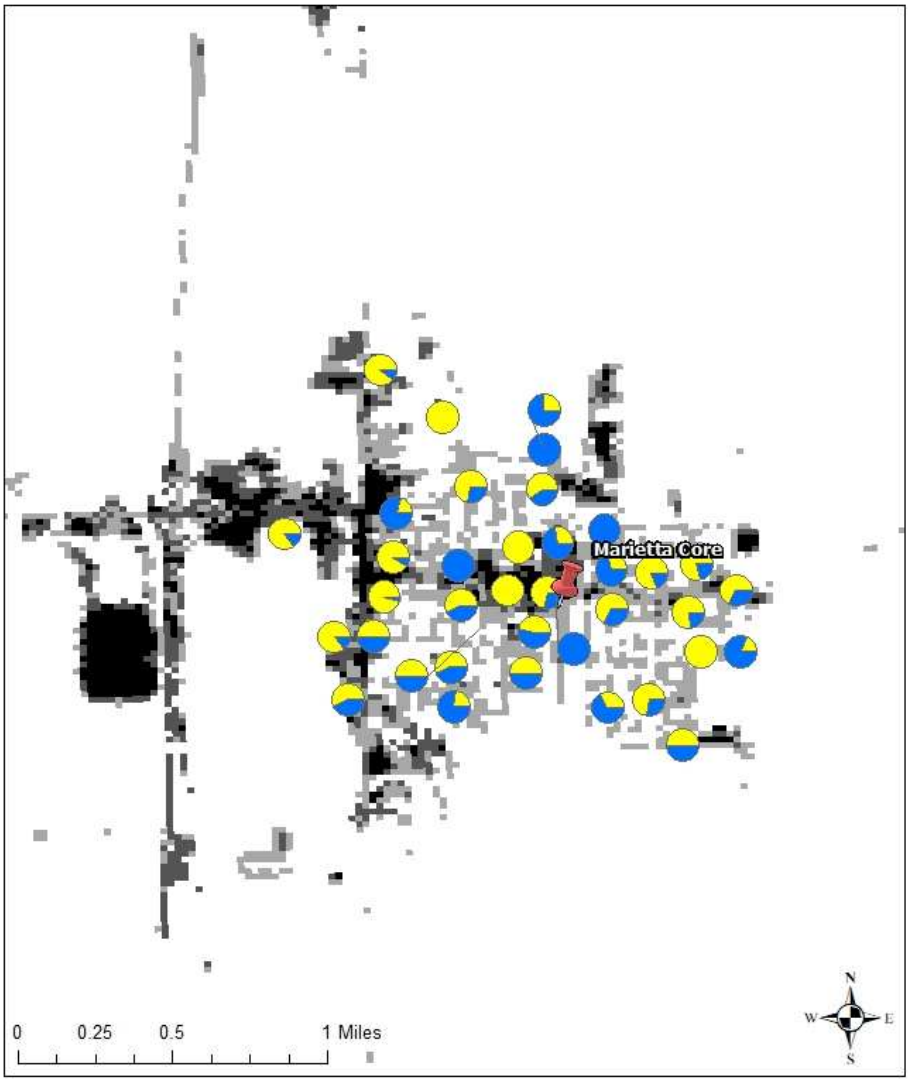
Impervious_Surface 2017_Mosquito_Data

VALUE

- 0 - 25
- 25.00000001 - 50
- 50.00000001 - 75
- 75.00000001 - 100

- albo_tot
- aeg_tot

Marietta, OK Mosquito Data and Impervious Surfaces



Legend

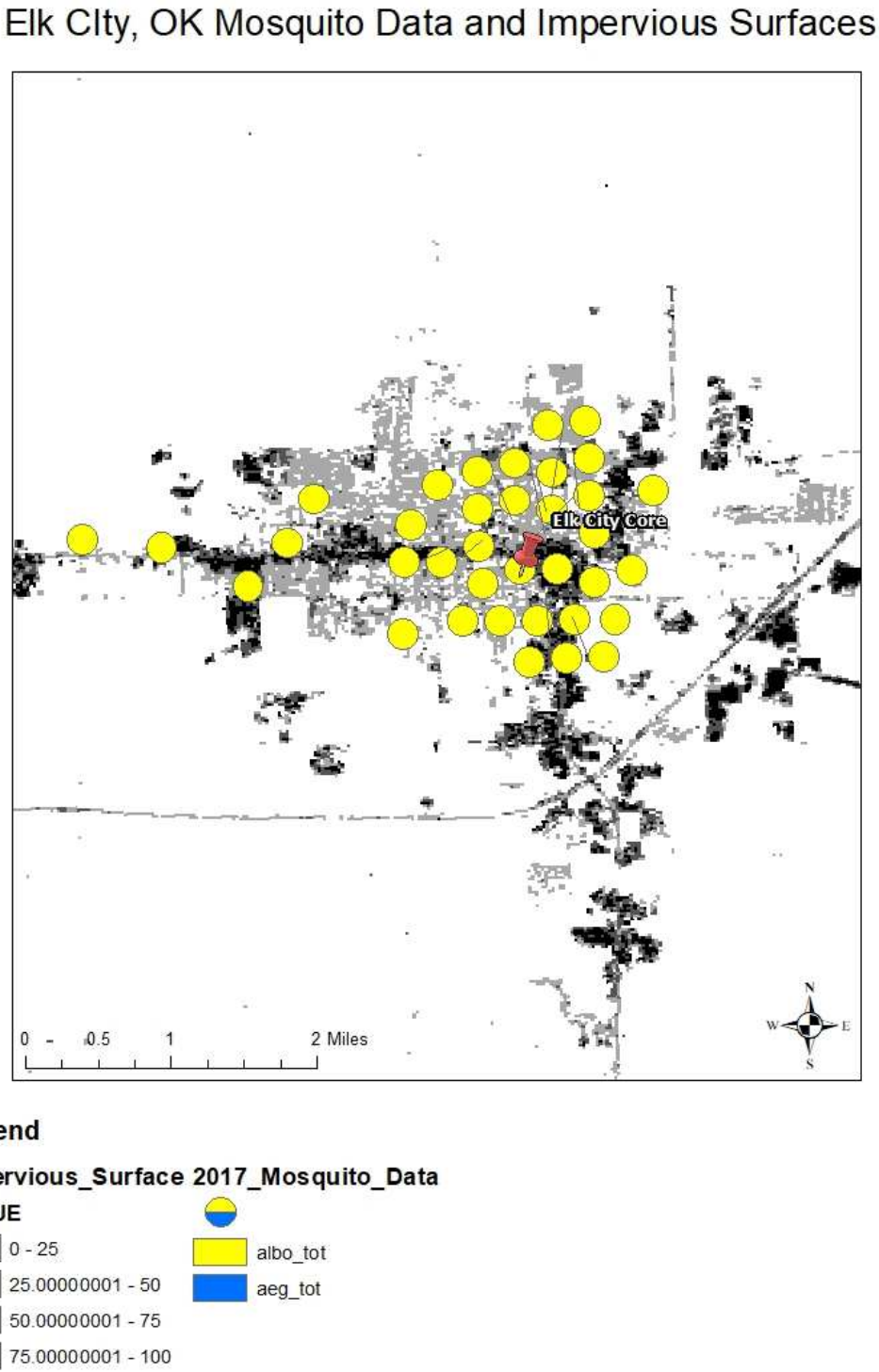
Impervious_Surface 2017_Mosquito_Data

VALUE

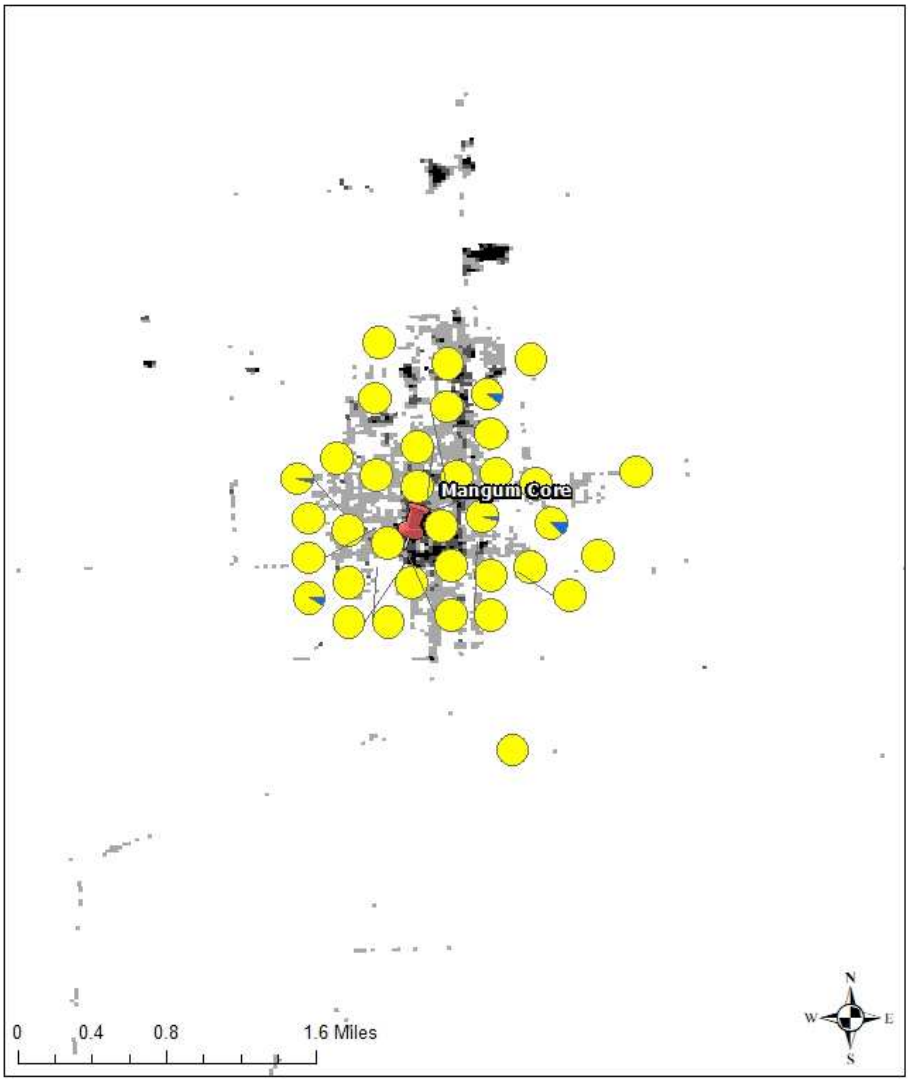
- 0 - 25
- 25.00000001 - 50
- 50.00000001 - 75
- 75.00000001 - 100

- albo_tot
- aeg_tot

Western Region Proportion Maps



Mangum, OK Mosquito Data and Impervious Surfaces



Legend

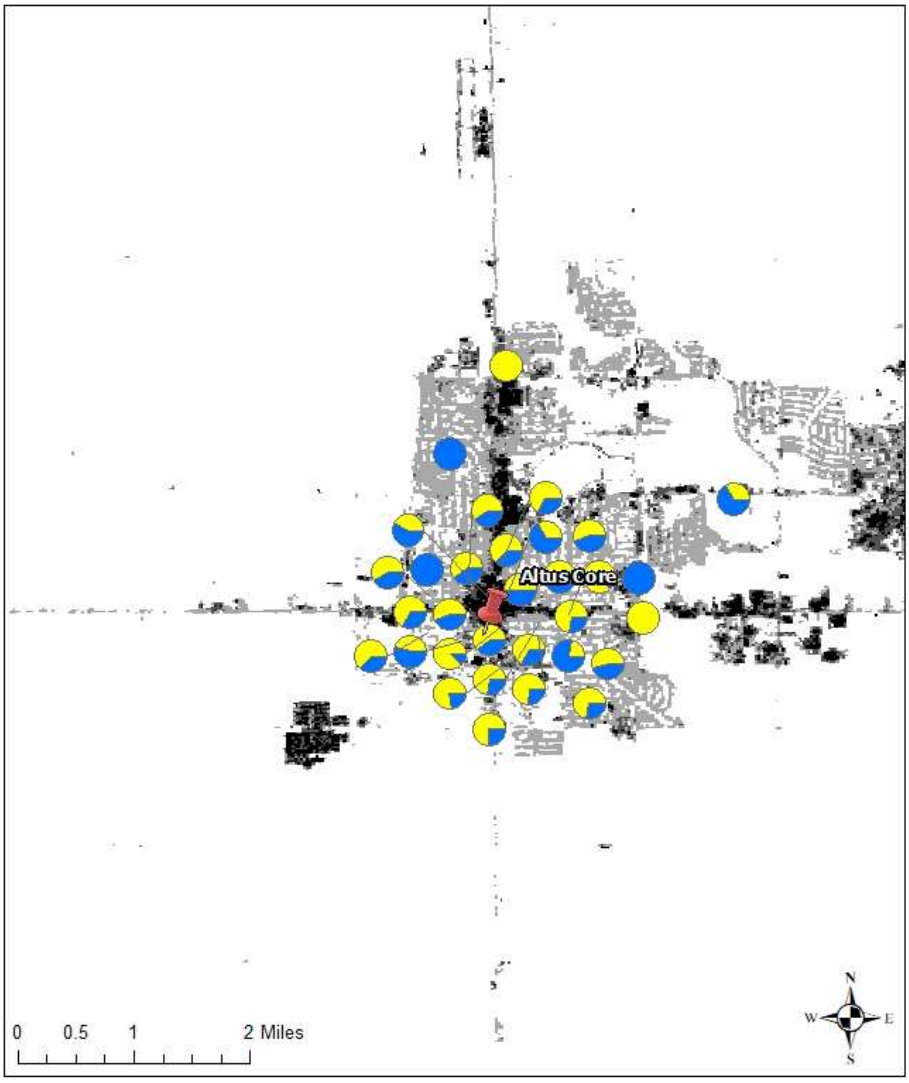
Impervious_Surface 2017_Mosquito_Data

VALUE

- 0 - 25
- 25.00000001 - 50
- 50.00000001 - 75
- 75.00000001 - 100

- albo_tot
- aeg_tot

Altus, OK Mosquito Data and Impervious Surfaces



Legend

Impervious_Surface 2017_Mosquito_Data

VALUE

- 0 - 25
- 25.00000001 - 50
- 50.00000001 - 75
- 75.00000001 - 100

- albo_tot
- aeg_tot

Appendix 6

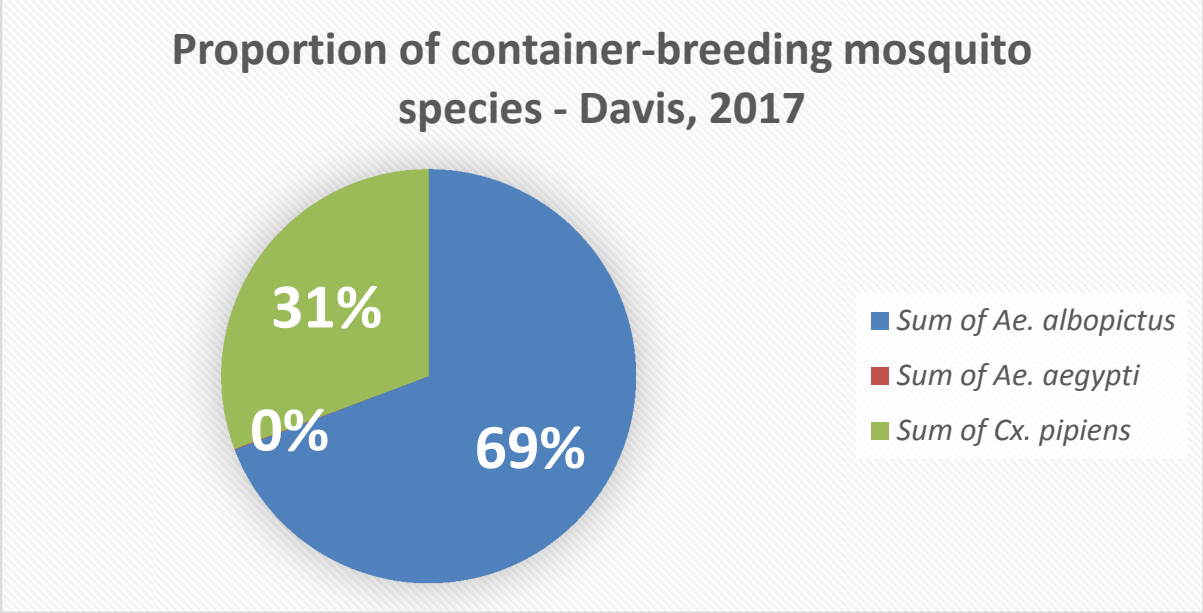
Extension Summaries 2017

Central Region Mosquito 2017 Summaries

Mosquitoes in Davis, OK, from May-August 2017

Report prepared by Jordan Sanders, Dr. Bruce Noden and Dr. Justin Talley

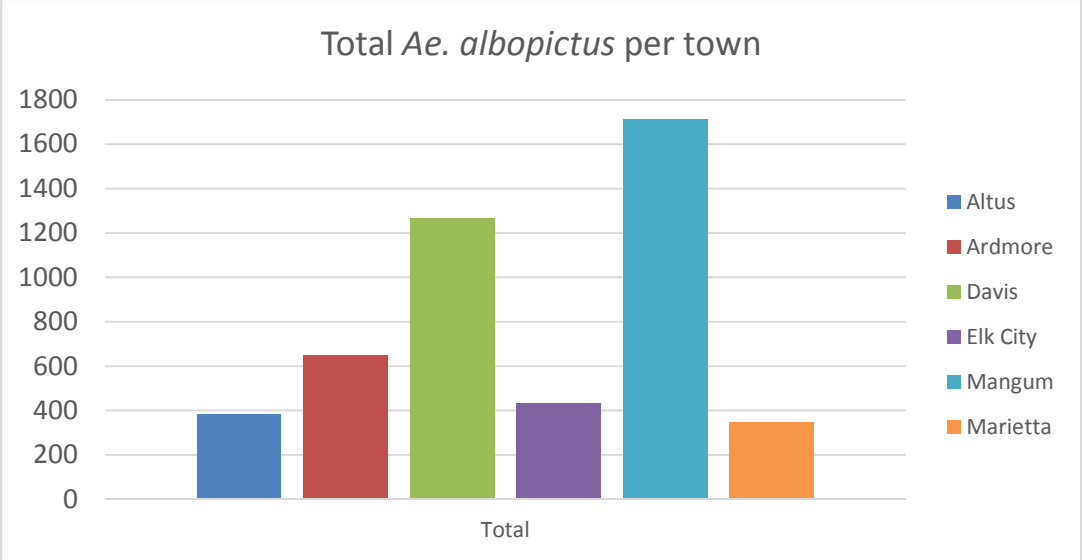
(Department of Entomology and Plant Pathology, Oklahoma State University)

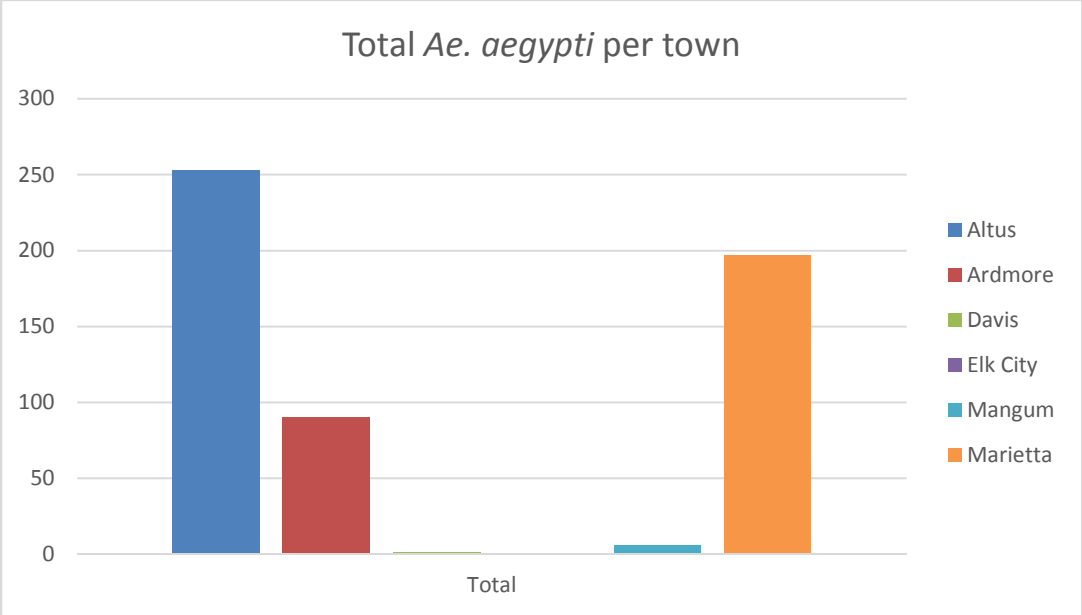


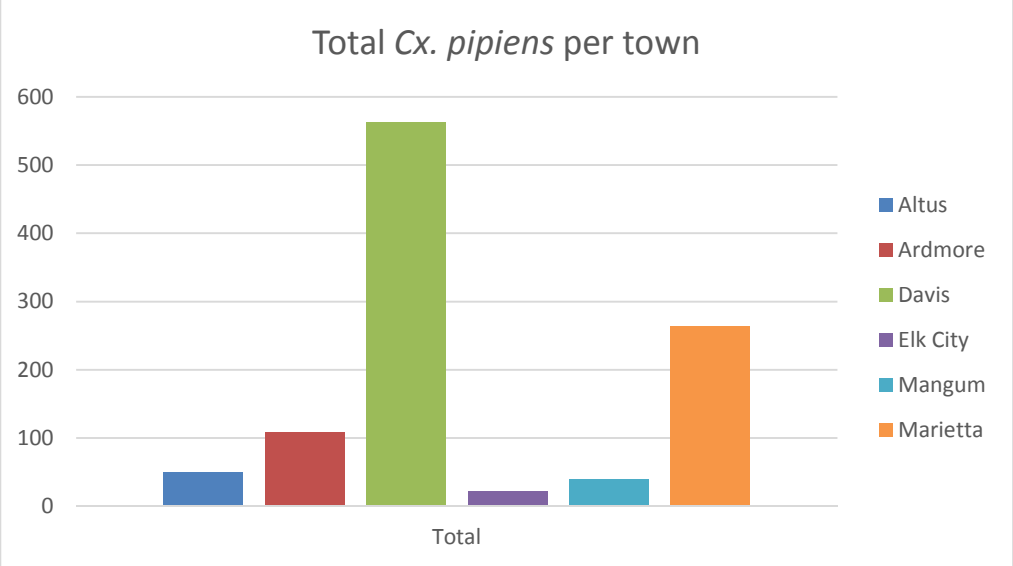
Main species collected in Summer 2017 were *Aedes albopictus* followed by *Culex pipiens* complex. We possibly found one *A. aegypti* which we are still working to confirm. By city, Davis had the highest numbers of *Culex pipiens* complex collected over the summer. There were also a high number of *A. albopictus* collected as well when compared with other cities.

- *Aedes albopictus* are container breeding mosquitoes which are vectors for dog heartworm and potential vectors for Dengue, Zika, and Chikungunya virus.
- *Culex pipiens/quinquefasciatus* breed in sewer areas or anywhere water can get nasty. They are the main vectors for West Nile virus in Oklahoma.

How did your town compare with other sites in Oklahoma?







Control Aspects to Consider:

- Both container breeding mosquitoes and transient / permanent water breeding mosquitoes were found which will need a comprehensive integrated control program
- *Aedes albopictus* are considered container breeders so the majority of control efforts need to be directed towards source reduction of water in containers in and around the property. Also, more education opportunities should be looked at so that individual property owners can assist with source reduction of water instead of city personnel trying to control these mosquitoes. Inspection of insect screens on windows should be emphasized to prevent these mosquitoes from entering the home.
- *Culex spp.* are active around sunset and sometimes just before sunrise and city personnel should have an active adulticide program with ULV applications combined with larviciding storm drains or drainage associated with rain runoff.

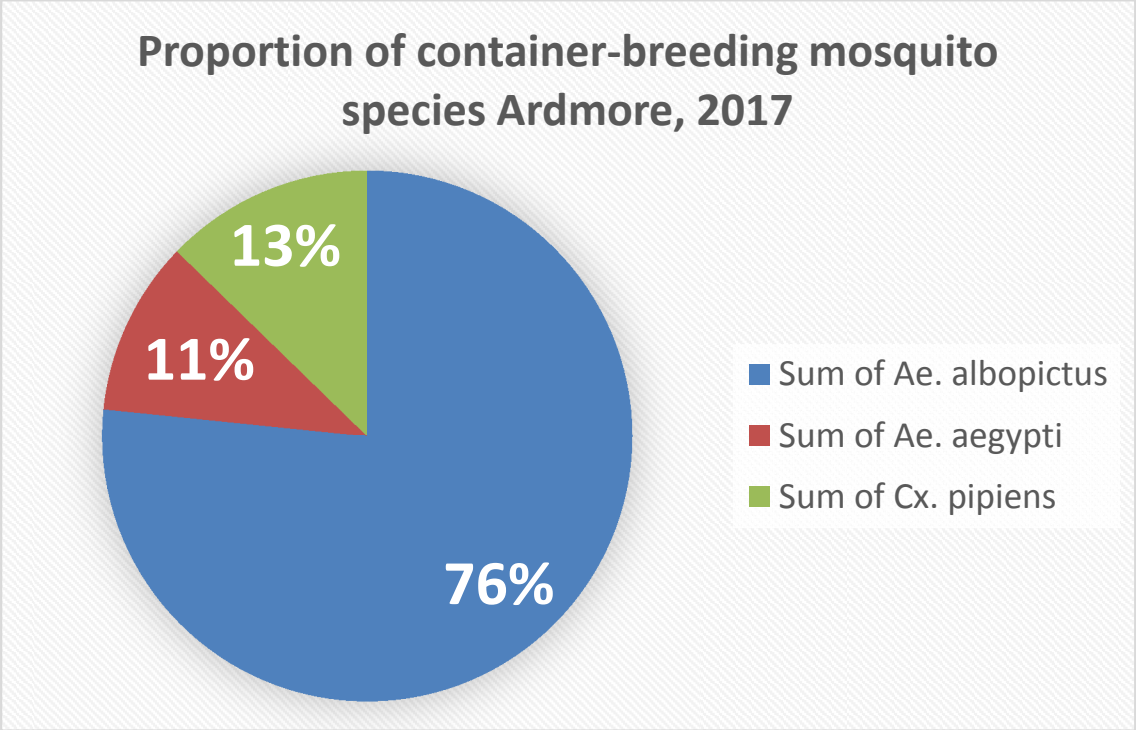
For more information please visit the Oklahoma State University Department of Entomology's mosquito information page at:
http://entoplp.okstate.edu/mosquito/mosquitoes

Acknowledgement of support: Funding for this project was provided by Centers for Disease Control and Prevention (CDC) through the Oklahoma Department of Health.

Mosquitoes in Ardmore, OK, from May-August 2017

Report prepared by Jordan Sanders, Dr. Bruce Noden and Dr. Justin Talley

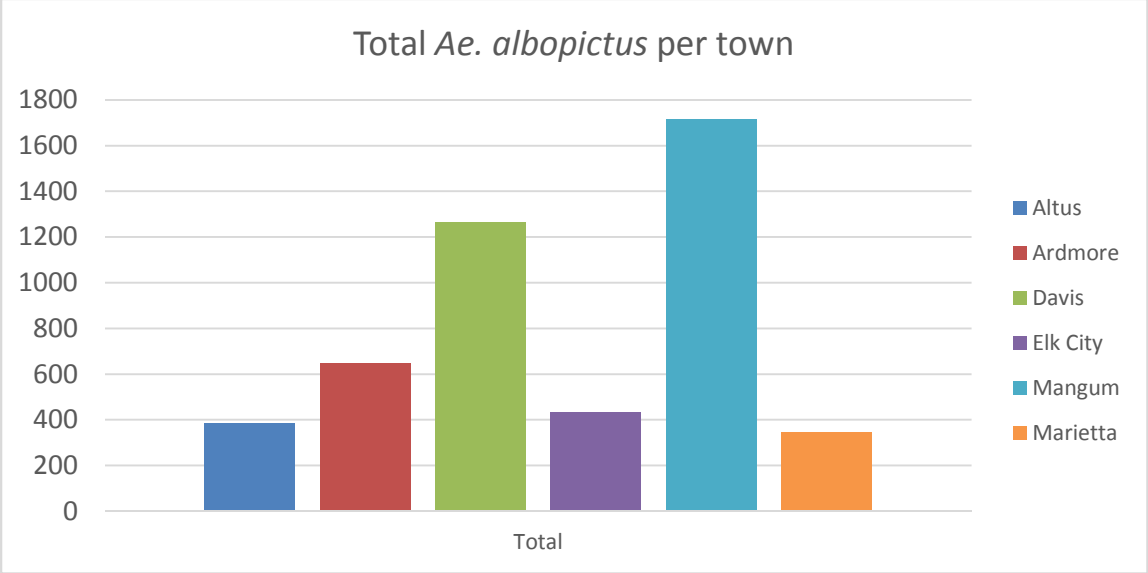
(Department of Entomology and Plant Pathology, Oklahoma State University)

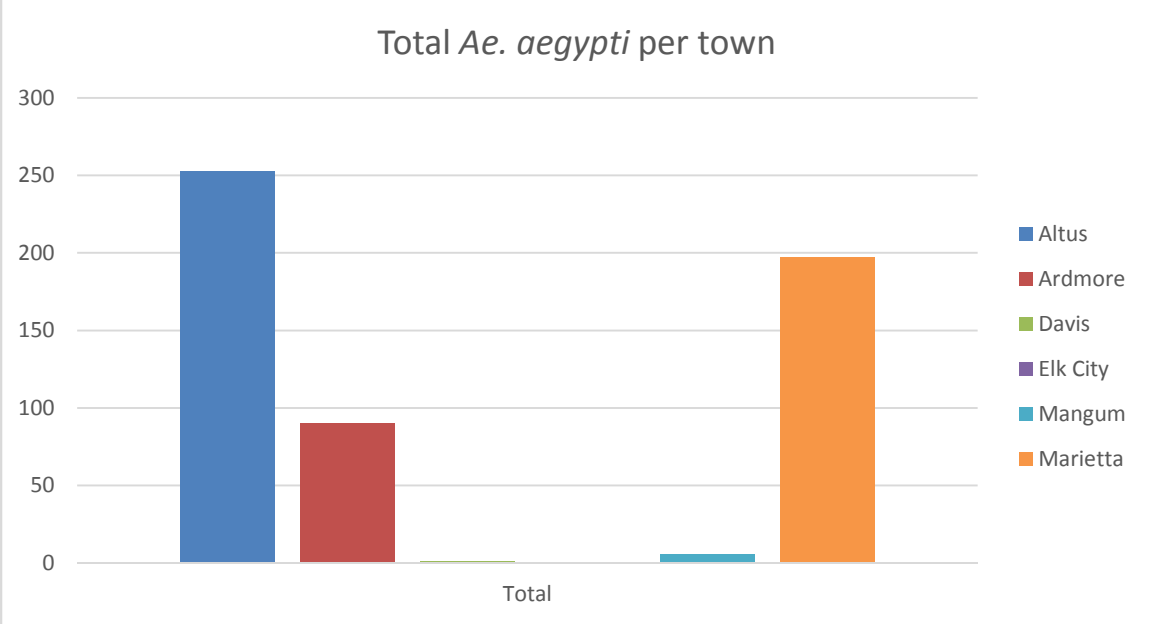


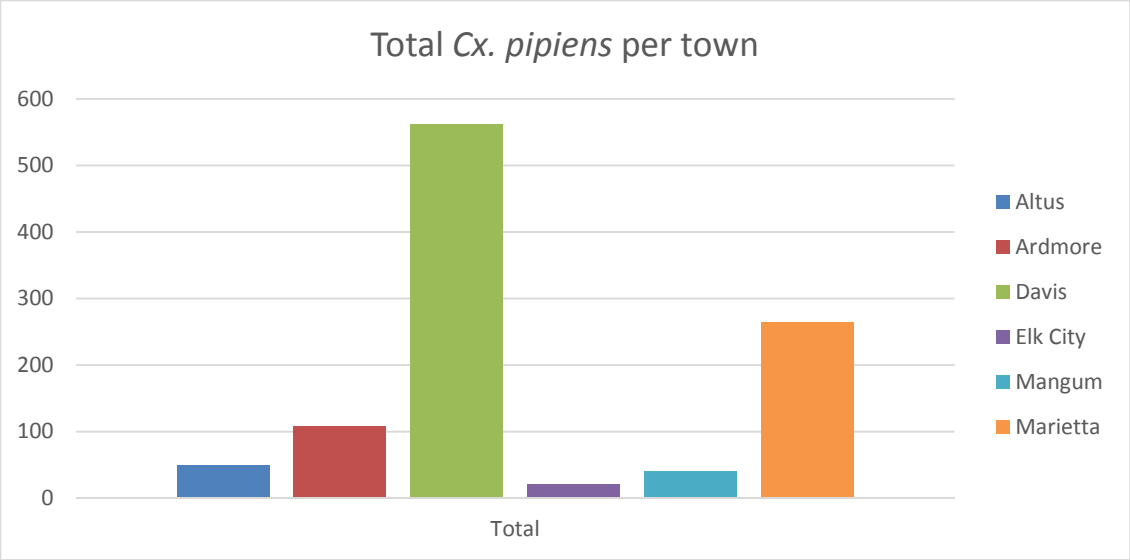
Main species collected in Summer 2017 were *Aedes albopictus* followed by *Culex pipiens* complex and *Aedes aegypti*. We found all three species spread throughout the whole urban core of Ardmore.

- *Aedes albopictus* and *A. aegypti* are container breeding mosquitoes which are vectors for dog heartworm and potential vectors for Dengue, Zika, and Chikungunya virus.
- *Culex pipiens/quinquefasciatus* breed in sewer areas or anywhere water can get nasty. They are the main vectors for West Nile virus in Oklahoma.

How did your town compare with other sites in Oklahoma?







Control Aspects to Consider:

- Both container breeding mosquitoes and transient / permanent water breeding mosquitoes were found which will need a comprehensive integrated control program
- *Aedes albopictus* and *Aedes aegypti* are considered container breeders so the majority of control efforts need to be directed towards source reduction of water in containers in and around the property. Also, more education opportunities should be looked at so that individual property owners can assist with source reduction of water instead of city personnel trying to control these mosquitoes. Inspection of insect screens on windows should be emphasized to prevent these mosquitoes from entering the home.
- *Culex spp.* are active around sunset and sometimes just before sunrise and city personnel should have an active adulticide program with ULV applications combined with larviciding storm drains or drainage associated with rain runoff.

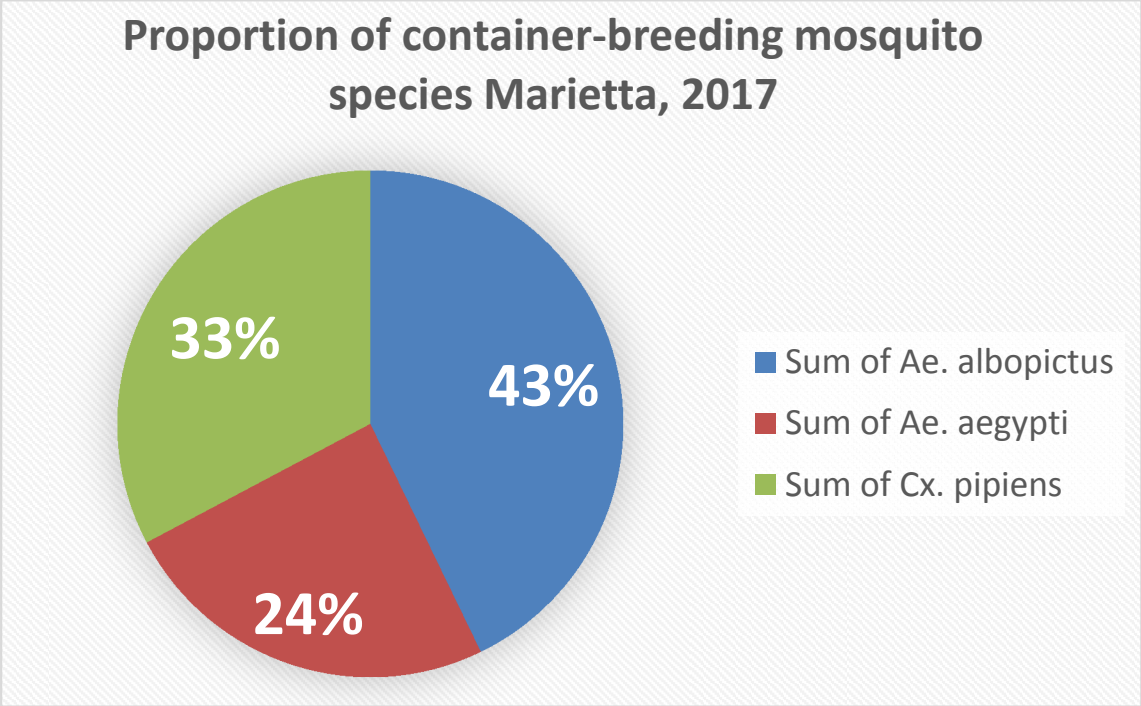
For more information please visit the Oklahoma State University Department of Entomology's mosquito information page at:
http://entoplp.okstate.edu/mosquito/mosquitoes

Acknowledgement of support: Funding for this project was provided by Centers for Disease Control and Prevention (CDC) through the Oklahoma Department of Health.

Mosquitoes in Marietta, OK, from May-August 2017

Report prepared by Jordan Sanders, Dr. Bruce Noden and Dr. Justin Talley

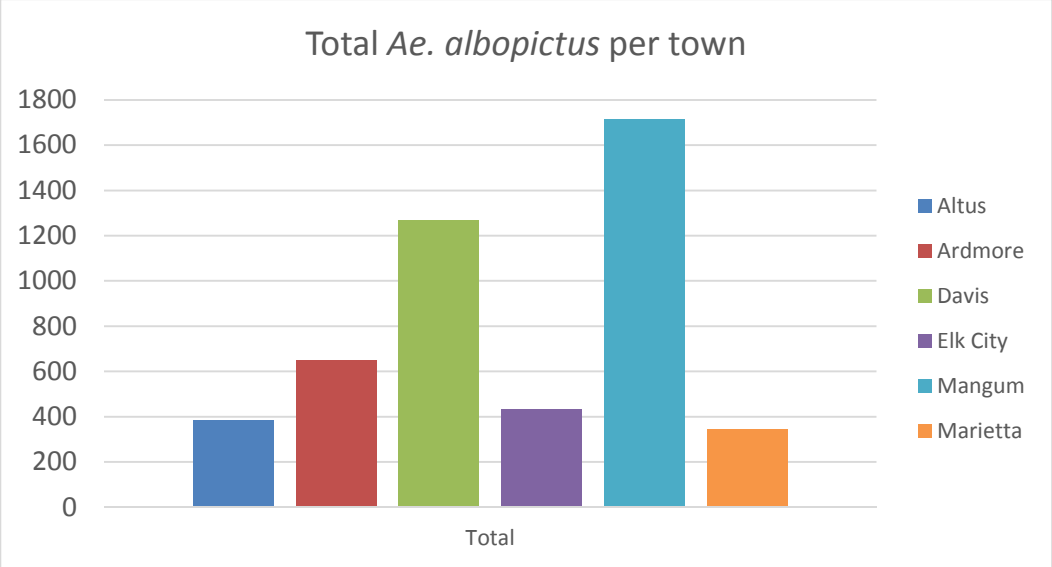
(Department of Entomology and Plant Pathology, Oklahoma State University)

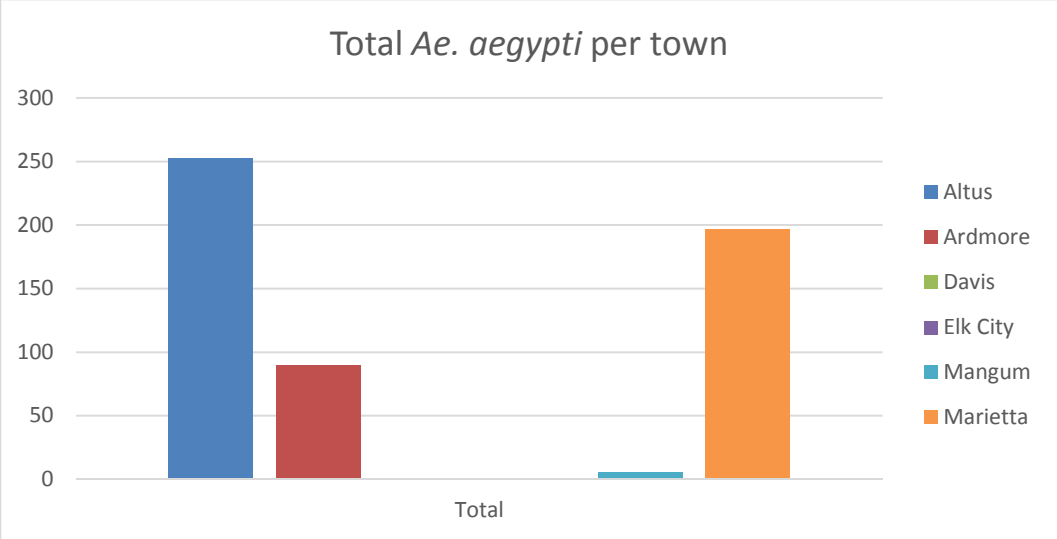


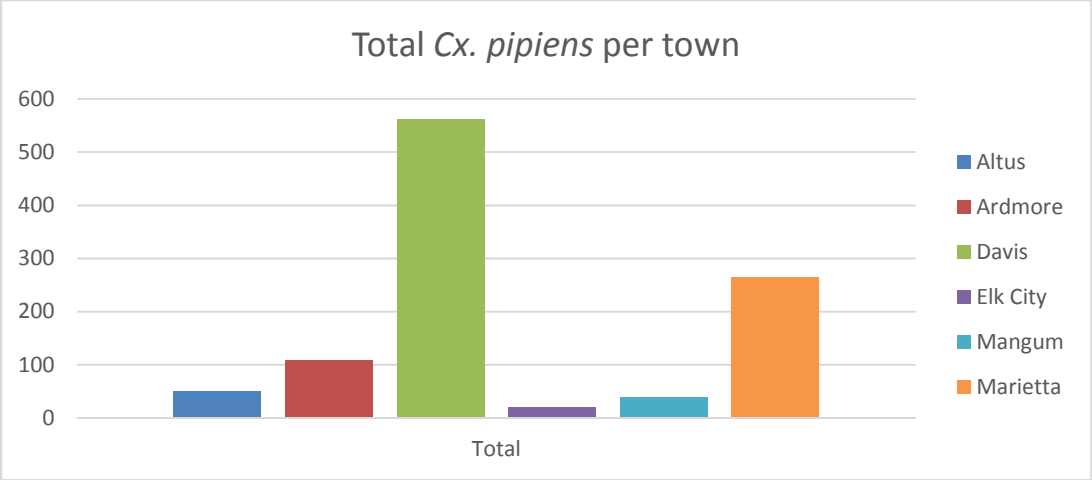
Main species collected in Summer 2017 were *Aedes albopictus* followed by *Culex pipiens* complex and *Aedes aegypti*. We found all three species spread throughout the whole urban core of Marietta. Compared with other cities, Marietta had the second highest number of *A. aegypti* collected.

- *Aedes albopictus* and *A. aegypti* are container breeding mosquitoes which are vectors for dog heartworm and potential vectors for Dengue, Zika, and Chikungunya virus.
- *Culex pipiens/quinquefasciatus* breed in sewer areas or anywhere water can get nasty. They are the main vectors for West Nile virus in Oklahoma.

How did your town compare with other sites in Oklahoma?







Control Aspects to Consider:

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- *Aedes albopictus* and *Aedes aegypti* are considered container breeders so the majority of control efforts need to be directed towards source reduction of water in containers in and around the property. Also, more education opportunities should be looked at so that individual property owners can assist with source reduction of water instead of city personnel trying to control these mosquitoes. Inspection of insect screens on windows should be emphasized to prevent these mosquitoes from entering the home.
- *Culex spp.* are active around sunset and sometimes just before sunrise and city personnel should have an active adulticide program with ULV applications combined with larviciding storm drains or drainage associated with rain runoff.

For more information please visit the Oklahoma State University Department of Entomology's mosquito information page at:
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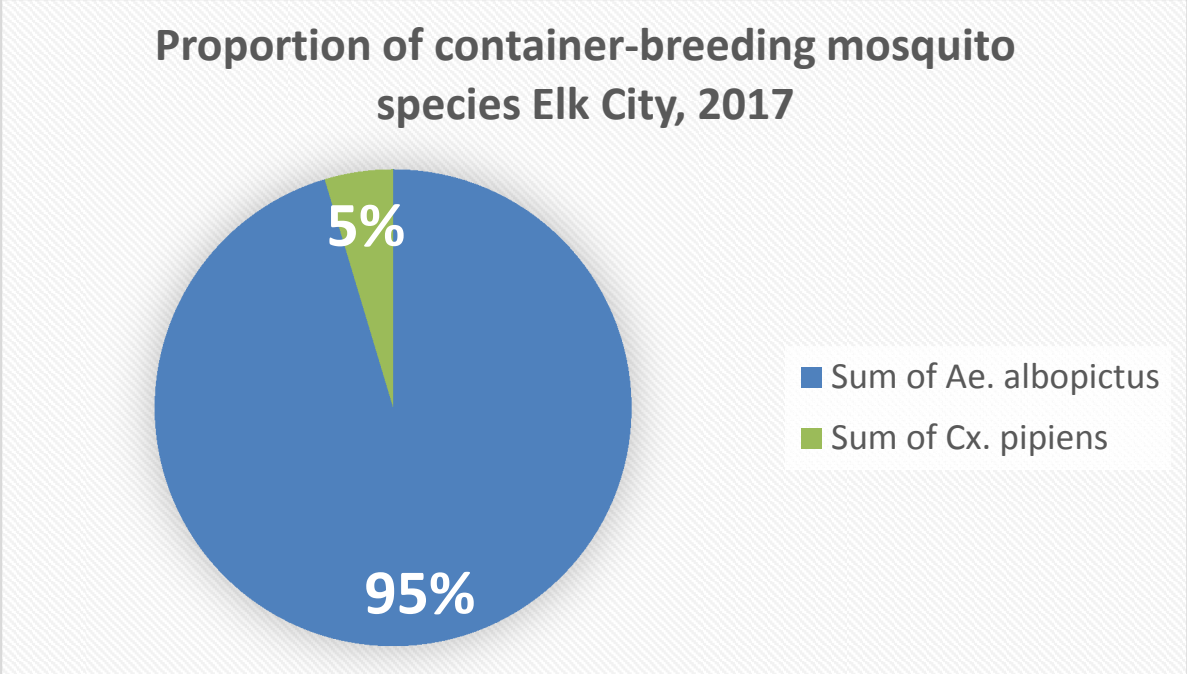
Acknowledgement of support: Funding for this project was provided by Centers for Disease Control and Prevention (CDC) through the Oklahoma Department of Health.

Western Region Mosquito 2017 Summaries

Mosquitoes in Elk City, OK, from May-August 2017

Report prepared by Jordan Sanders, Dr. Bruce Noden and Dr. Justin Talley

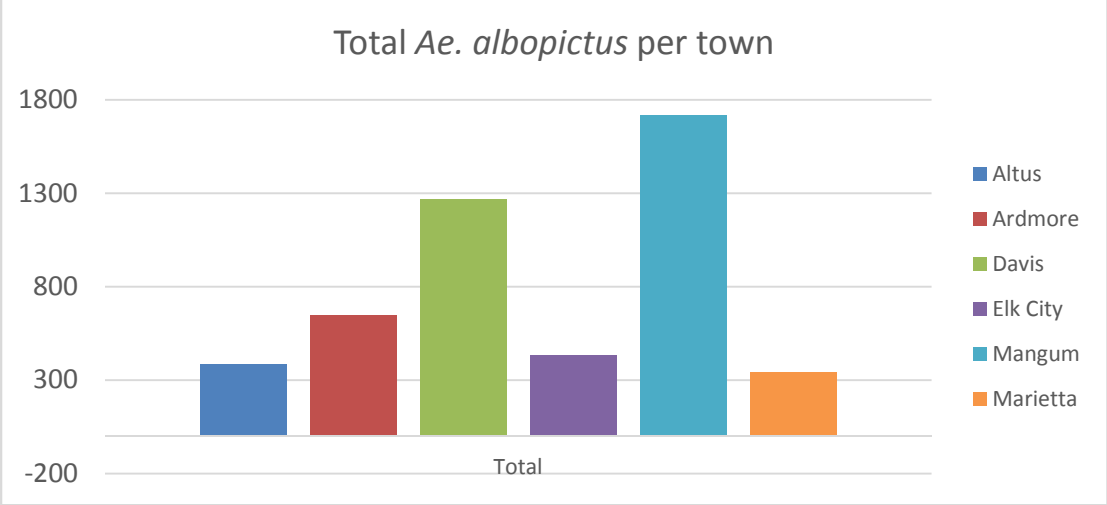
(Department of Entomology and Plant Pathology, Oklahoma State University)

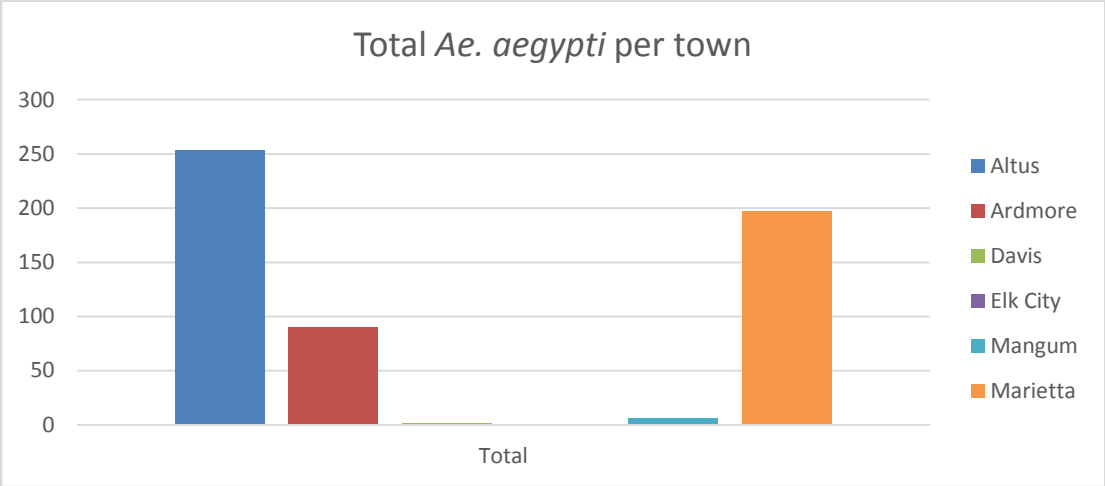


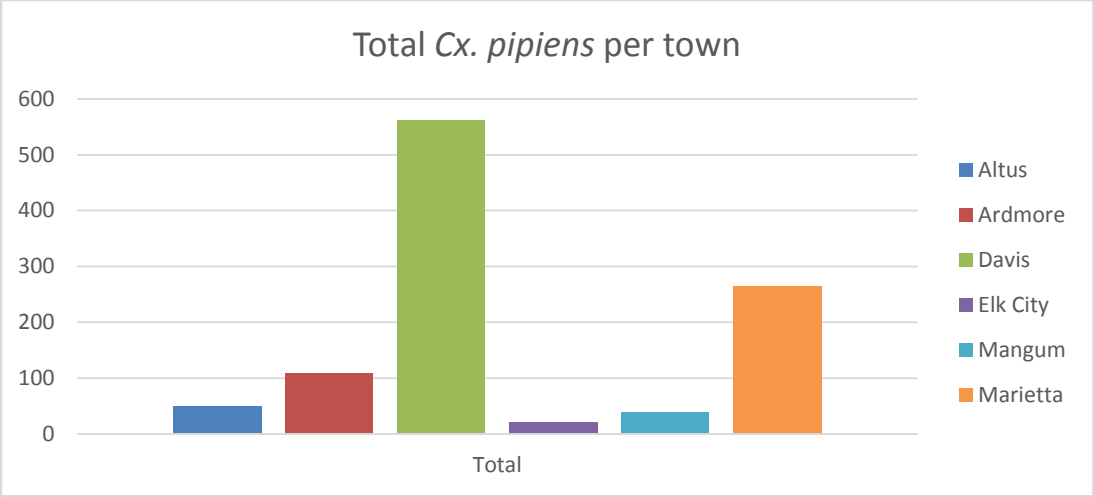
Main species collected in Summer 2017 were *Aedes albopictus* followed by *Culex pipiens* complex.

- *Aedes albopictus* are container breeding mosquitoes which are vectors for dog heartworm and potential vectors for Dengue, Zika, and Chikungunya virus.
- *Culex pipiens/quinquefasciatus* breed in sewer areas or anywhere water can get nasty. They are the main vectors for West Nile virus in Oklahoma.

How did your town compare with other sites in Oklahoma?







Control Aspects to Consider:

- Both container breeding mosquitoes and transient / permanent water breeding mosquitoes were found which will need a comprehensive integrated control program
- *Aedes albopictus* are considered container breeders so the majority of control efforts need to be directed towards source reduction of water in containers in and around the property. Also, more education opportunities should be looked at so that individual property owners can assist with source reduction of water instead of city personnel trying to control these mosquitoes. Inspection of insect screens on windows should be emphasized to prevent these mosquitoes from entering the home.
- *Culex spp.* are active around sunset and sometimes just before sunrise and city personnel should have an active adulticide program with ULV applications combined with larviciding storm drains or drainage associated with rain runoff.

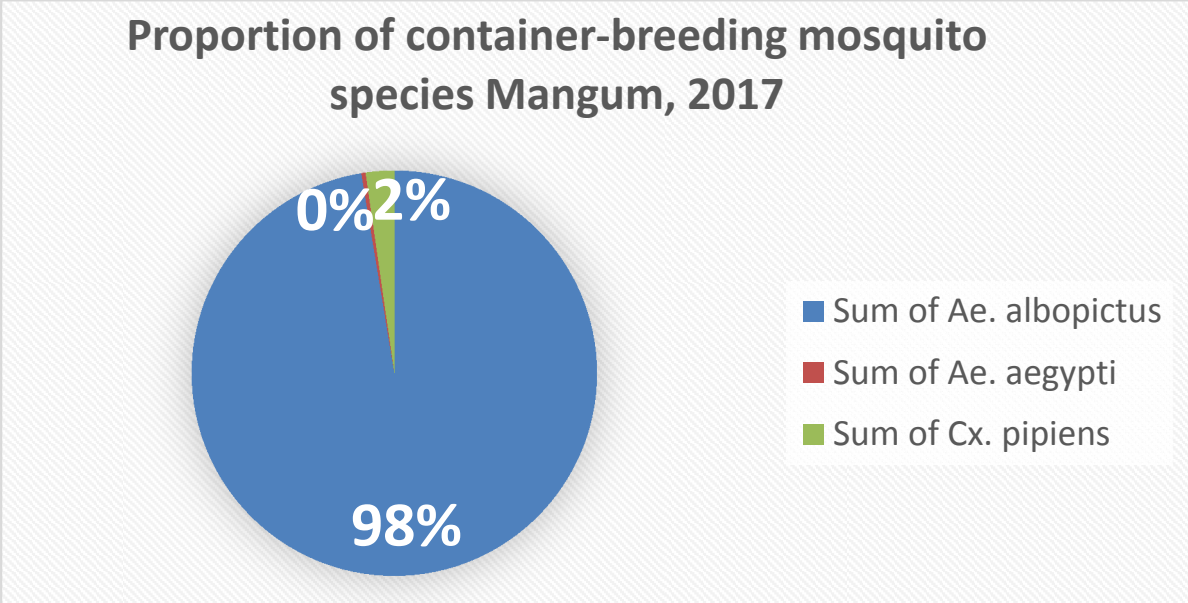
For more information please visit the Oklahoma State University Department of Entomology's mosquito information page at:
http://entoplp.okstate.edu/mosquito/mosquitoes

Acknowledgement of support: Funding for this project was provided by Centers for Disease Control and Prevention (CDC) through the Oklahoma Department of Health.

Mosquitoes in Mangum, OK, from May-August 2017

Report prepared by Jordan Sanders, Dr. Bruce Noden and Dr. Justin Talley

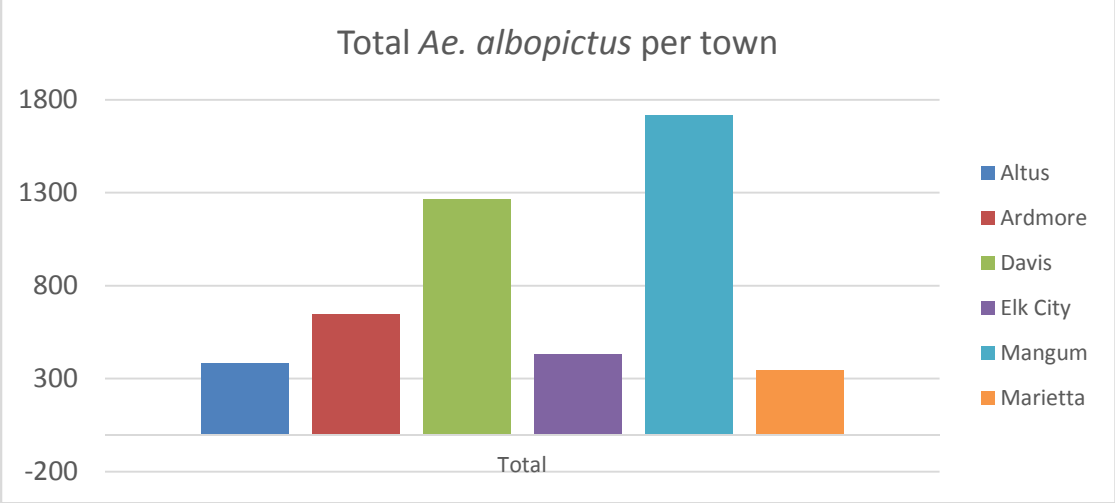
(Department of Entomology and Plant Pathology, Oklahoma State University)

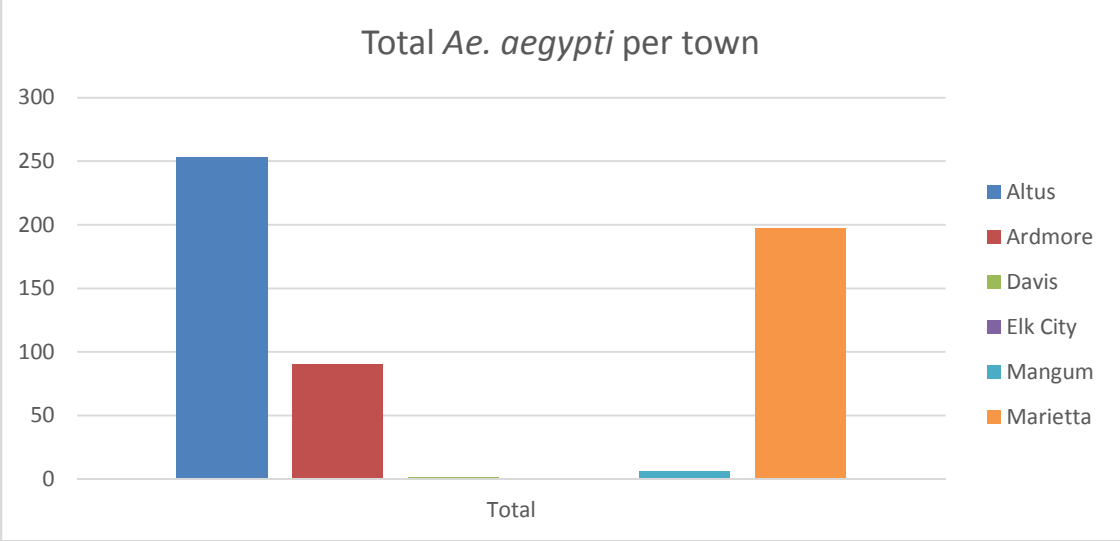


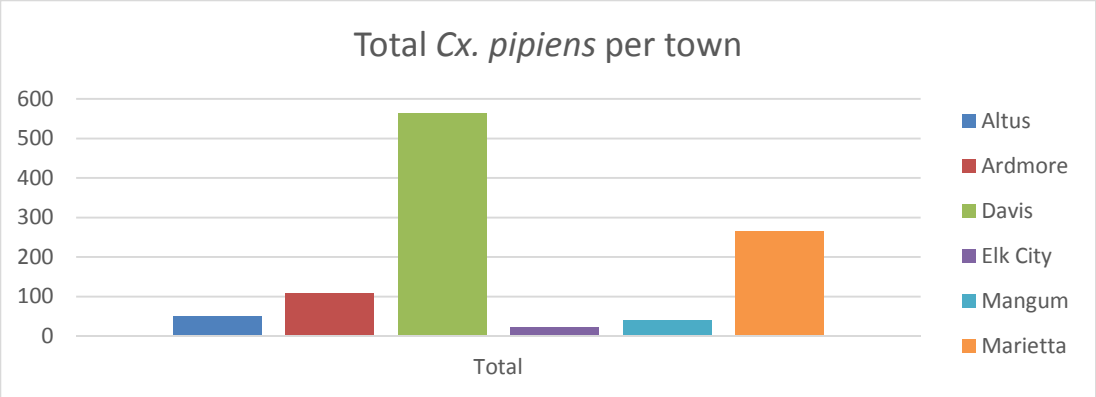
Main species collected in Summer 2017 were *Aedes albopictus* followed by *Culex pipiens* complex. By city, Mangum had the highest numbers of *A. albopictus* collected over the summer. We possibly found a few *A. aegypti* which we are still working to confirm.

- *Aedes albopictus* are container breeding mosquitoes which are vectors for dog heartworm and potential vectors for Dengue, Zika, and Chikungunya virus.
- *Culex pipiens/quinquefasciatus* breed in sewer areas or anywhere water can get nasty. They are the main vectors for West Nile virus in Oklahoma.

How did your town compare with other sites in Oklahoma?







Control Aspects to Consider:

- Both container breeding mosquitoes and transient / permanent water breeding mosquitoes were found which will need a comprehensive integrated control program
- *Aedes albopictus* and *Aedes aegypti* are considered container breeders so the majority of control efforts need to be directed towards source reduction of water in containers in and around the property. Also, more education opportunities should be looked at so that individual property owners can assist with source reduction of water instead of city personnel trying to control these mosquitoes. Inspection of insect screens on windows should be emphasized to prevent these mosquitoes from entering the home.
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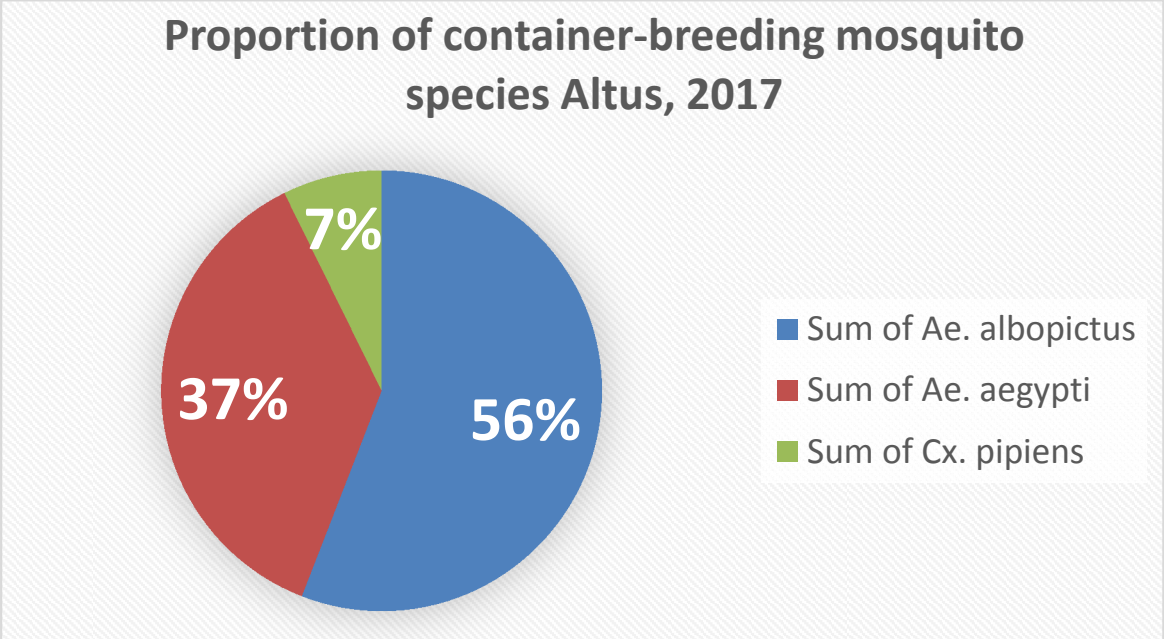
Acknowledgement of support: Funding for this project was provided by Centers for Disease Control and Prevention (CDC) through the Oklahoma Department of Health.

Mosquitoes in Altus, OK, from May-August 2017

Report prepared by Jordan Sanders, Dr. Bruce Noden and Dr. Justin Talley

(Department of Entomology and Plant Pathology, Oklahoma State University)



Main species collected in Summer 2017 were *Aedes albopictus* followed by *Aedes aegypti* and *Culex pipiens* complex. We found all three species spread throughout the whole urban core of Altus. By city, Altus had the highest numbers of *A. aegypti* collected over the summer.

- *Aedes albopictus* and *A. aegypti* are container breeding mosquitoes which are vectors for dog heartworm and potential vectors for Dengue, Zika, and Chikungunya virus.
- *Culex pipiens/quinquefasciatus* breed in sewer areas or anywhere water can get nasty. They are the main vectors for West Nile virus in Oklahoma.

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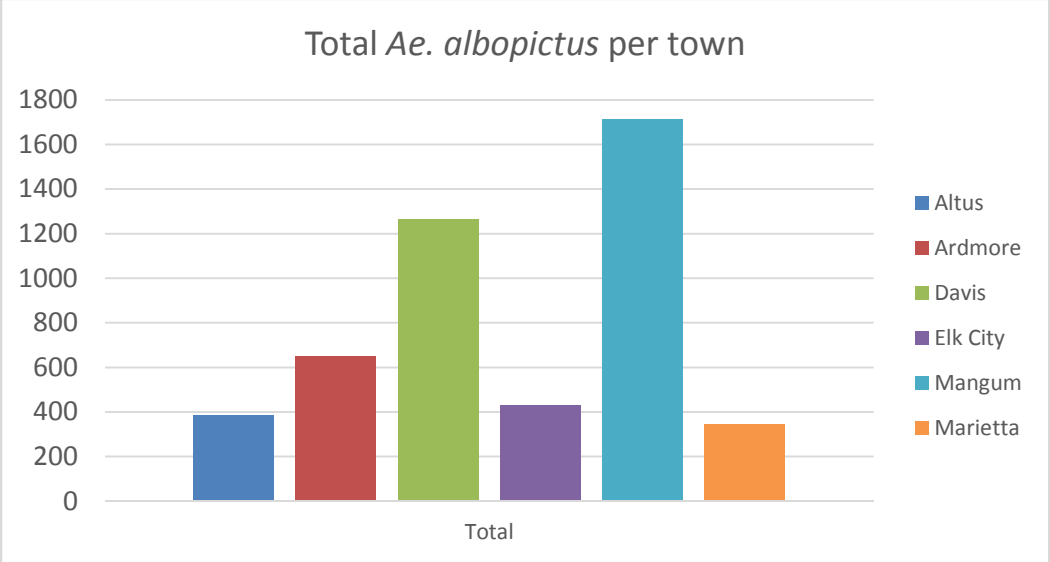

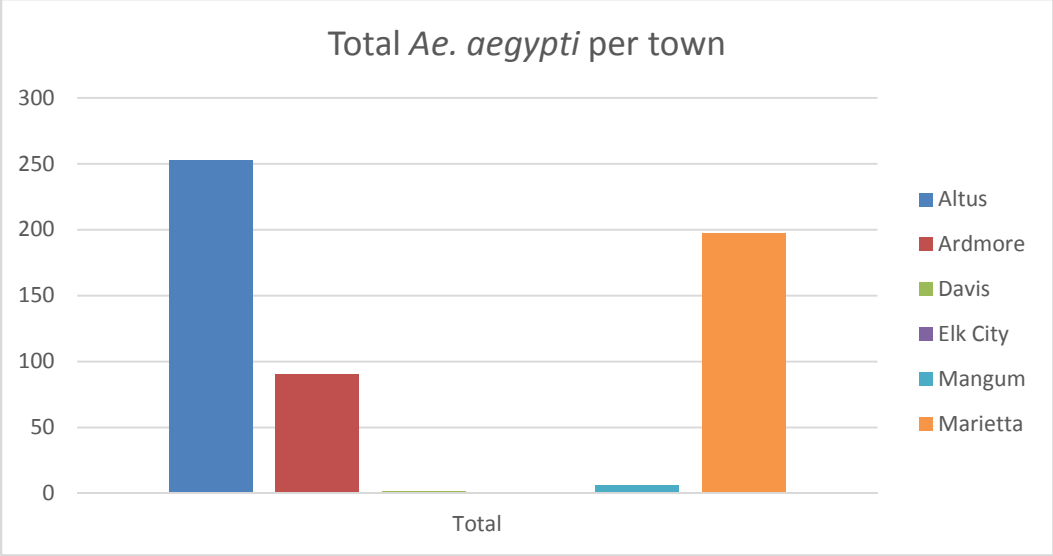

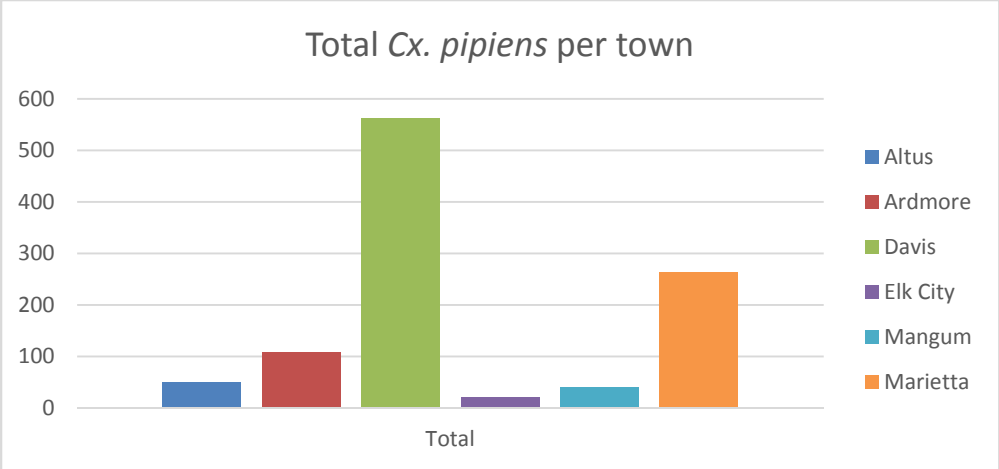

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VITA

JORDAN DANE'E SANDERS

Candidate for the Degree of

Master of Science

Thesis: DISTRIBUTION OF CONTAINER-BREEDING MOSQUITO SPECIES IN URBAN AREAS OF SOUTHERN OKLAHOMA

Major Field: Entomology and Plant Pathology

Education:

December 2018. Master of Science, Entomology and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma.
May 2016. Bachelor of Science in Animal Science, Oklahoma State University, Stillwater, Oklahoma.

Professional Presentations:

Sanders, J, Noden, BH. 2018. *Habitat preferences of container breeding mosquito populations in urban areas in southern Oklahoma*. Presentation by JS at Southwest Branch ESA meeting, Albuquerque, New Mexico (March 25-29, 2018).
Sanders, J, Noden, BH. 2017. *The distribution of container breeding mosquito species in urban areas of southern Oklahoma*. Presentation by JS at the National ESA meeting, Denver, CO (Nov 2017).

Experience:

2017-present. Oklahoma State University: Department of Entomology and Plant Pathology.
Graduate Research Assistant and Graduate Teaching Assistant
- Surveillance of container breeding mosquito species in urban areas of southern Oklahoma

2016-2017 Oklahoma State University Department of Entomology and Plant Pathology.
Undergraduate Teaching Assistant