EASTERN RED CEDAR (*JUNIPERUS VIRGINIANA*) EXPANSION RELATED TO WEST NILE VIRUS TRANSMISSION BY *CULEX* SPECIES IN OKLAHOMA LIVESTOCK OPERATIONS

By

COURTNEY MAICHAK

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EASTERN RED CEDAR (*JUNIPEROUS VIRGINIANA*)

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Thesis Approved:

Dr. Bruce Noden

Thesis Adviser

Dr. Justin Talley

Dr. Scott Loss

Dr. Kris Hiney

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Name: COURTNEY MAICHAK

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Abstract: Eastern Redcedar (*Juniperus virginiana*) (ERC) is a native invader in the United States Great Plains. While native, it has expanded beyond its range and is rapidly invading pastures throughout the southern Great Plains. Concurrently with the ERC invasion, West Nile Virus (WNV), transmitted mainly by *Culex* mosquitoes, has become a significant mosquito-borne disease in the Great Plains region since its introduction into the United States in 1999. To date, few studies have evaluated the association of ERC expansion with the risk for WNV in the southern Great Plains. Given these aspects of ERC expansion and the need to focus on *Culex* sp. in the region, the aim of our study was to evaluate how varying concentrations of ERC in different expansion areas impact mosquito populations in Oklahoma. To focus on these relationships, we tested the following hypotheses: 1) The abundance of mosquito communities is influenced by increasing concentrations of ERC in different regions of the state. 2) WNV-infected mosquitoes will be more likely to be collected in ERC than in grassland. To test our hypotheses, we collected mosquitoes in 32 different sites in 7 different ERC sites in 4 ERC expansion zones. We collected mosquitoes using CDC Light traps baited with CO_2 in addition to CDC gravid traps, 32-gallon bucket traps, wire-frame shelter traps, and fiber pot traps for collecting resting blood-fed mosquitoes. Based on mosquito collections in four ERC expansion areas in Oklahoma, we found that mosquito abundance is influenced by differing concentrations of ERC. Abundance of *Aedes albopictus* was directly related to higher concentrations of ERC while *Ps. columbiae* abundance was inversely related to increasing ERC concentration. The only impact of ERC on *Cx. tarsalis* occurred in western Oklahoma in Blaine County with more *Cx. tarsalis* collected in ERC than the grassland control site. Secondly, the only WNV-infected pool of mosquitoes detected were *Cx. tarsalis* collected in ERC. CDC light traps were more successful at collecting blood-fed and gravid mosquitoes than other methods. Overall, the results of the study indicate different uses of varying ERC concentrations by important mosquito vectors of WNV in the southern Great Plains region in the United States. These relationships may be important in gaining a better understanding of the risk of this important disease in this endemic region.

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CHAPTER I

REVIEW OF THE LITERATURE

Introduction

The United States Great Plains is a vast section of the central US that includes the area from North Dakota south into Central Texas. This region consisted of tall grass, short grass, and mixed prairies before the arrival of the settlers, in early 1600s from Spanish conquistadors and later European settlers in the late 1700s, who transformed the landscape into farms and pastures. This process of the landscape being manipulated from plowing, crop production, and eventually the Dust Bowl in the 1930s brought significant changes for the whole region. The Dust Bowl brought encouragement from United States Department of Agriculture to plant Eastern Redcedar to be used as soil control and wind breaks. Settlement also brought fire suppression from the new inhabitants and the loss of soil structure brought considerable negative change to the region. These changes lead to the focus of this project to look at two threats in the Great Plains. The first threat is a native invader, the Eastern Redcedar (*Juniperus virginiana*) (ERC). This woody plant has always existed in the Great Plains, however, has become an increasing problem since Spanish and European settlement of the region. The cessation of wildfire controls on the prairies to preserve homesteads and farmland helped to increase the threat of ERC expansion westward and northward into new areas (Zou et al. 2018). ERC changes the environment around it by changing biogeochemical cycles in particular with water movement, such as evapotranspiration, runoff, and water recuperation (Zou et al. 2018). The second threat in the Great Plains is West Nile Virus (WNV) that, did not become a problem until it entered the United States in 1999. The

introduction of WNV into the country started in New York and only took four years to reach the Western border in California. Since then, the virus has become endemic and has remained an epidemic threat since its invasion. The pathogens success comes from the mosquito vectors that continually spread and transmit the virus to new hosts. Making them an important factor to consider when understanding the natural nidality, the pathogens capability to survive in the ecosystem.

Eastern Red Cedar Invasion

Eastern red cedar (*Juniperus virginiana*, Linnaeus) (ERC) is a native woody evergreen tree in the Eastern and central parts of the United States making it one of the most widely distributed species (Van Haverbeke and Read 1976). For the past several decades ERC has been invading the United States Great Plains (Brag and Hulbert 1976). In this region the primary ecosystem is the grassland, with approximately 30% of the historical range still remaining, and less than 10% of the tallgrass ecosystem still natural (Samson and Knopf 1994; White et al. 2000). Due to it being native in the Great Plains, it can be found in both tallgrass and shortgrass prairies (Engle and Kulbeth 1992).

This juniper encroachment has been attributed to many reasons, but a major factor is due to the species naturally occurring in these areas. ERC has population characteristics that favor its invasion, including rapid maturity, high fecundity, and vast dispersal from hosts such as ungulates and birds (Holthuizjen and Sharik 1985). These factors play important roles in its ability to spread rapidly and widely, with agricultural practices being one of the strongest reasons for the landscape change. These land use changes have been a major cause to ERC establishing in the Great Plains region. One of the first alterations made to the land was fencing, to provide defined areas of land to graze livestock, thus creating land segmentation. Grazing of native species has intensive effects to disturbing the landscape and halting invasive ERC establishment (Schmidt and Stubbendieck 1993; Scholes and Archer 1997). These areas with heavy grazing by the cattle

controlled the ERC, however, some ERC success came from the natural ungulates being pushed out of their native ranges. This provided an easier pathway for ERC to expand its range. Another factor that allowed ERC to spread was controlling wildfires and decreased prescribed fires that would normally destroy the trees (Blewett 1984; Briggs et al 2002). According to Wright and Bailey (1982) the ERC is highly fire sensitive and would normally be removed by the by naturally occurring fires. Native Americans also practiced prescribed burns to improve the landscape for the animals they hunted, such as deer and bison. One of the last major landscape changes made that was key to ERC expansion was the response to the Dust Bowl in the 1930s. ERC was used to help with soil erosion by stabilizing the ground layers and block wind. The decreased use of the two natural controls, fire and grazing, were essential to preventing the ecosystem from transitioning from the natural grassland to timberland (Twidwell et al. 2014; Ratajczak 2014). However, with a less intensive control systems in place and the combination of ERC use for erosion control it has been allowed to flourish and expand its range. Without removal of ERC and other trees, grasslands have been completely taken over with complete coverage of canopies (Brag and Hulbert 1976; Hoch and Briggs 1999). These grassland ecosystems can be taken over in under 40 years by juniper invaders (Briggs et al. 2002). Without control and loss of the grasslands, this directly impacts livestock grazing as well (Bernardo et al. 1988). This shift has created major changes in the natural ecosystem, particularly in Oklahoma.

Much of central Oklahoma is in the cross timbers portion of the United States where there is a mix of the native prairies from the West and the encroaching forests from the East. In this zone, oak trees have already invaded waterways (Abrams 1986; Knight et al. 1994). ERC is expected to be more of a nuisance since it is native and able to establish in areas that would typically be inhabitable for other species of trees (Owensby et al. 1973; Engle and Kulbeth 1992), including natural water drainages, hill tops, and drier areas. ERC have been expanding rapidly in ten counties in the central and western portions of the state, west of the crosstimbers region

(Wang et al. 2017, 2018). This could lead to competition for resources for the other native plants and animals that inhabit these areas.

An invasive species such as ERC contributes to substantial ecological change which result in several concerns. These general changes include a decrease in species richness, a decrease in species diversity, altered habitats, and a shift in the biogeochemical cycles (Broadfoot 1951; Meiners et al. 2001; Norris et al. 2001; Briggs et al. 2002; Siemann and Rogers 2003; Reihnhart et al. 2006; Zou et al. 2014; Zou et al. 2018). The altered water cycle is a possible concern in the drier climates that ERC has been establishing. This includes transpiration changes, with the average daily use of water from ERC being 24L per tree (Caterina et al. 2014), and surface runoff having an 80% reduction from grassland to ERC cover (Zou et al 2014). These are alterations that are made from non-native invaders and are also expected form native invaders such as ERC (Didham et al. 2005). The change from open grassland to closed canopy coverage alters the detritus layer, light coverage, and rain diversion (Engle et al. 1987; Smith and Stubbendieck 1990; Gehring and Bragg 1992). In these closed canopy areas, there has been up to 83% reduction of understory coverage from ERC invading into the open prairie ecosystems (Bard 1952; Engle et al. 1987; Smith and Stubbendieck 1990; Gehring and Bragg 1992). Even with removal and restoration, recovery of the natural grasslands can take three years with areas in high density ERC (>40%) (Ansley and Rasmussen 2005). The amount of time and effort it takes for ERC to be removed in the ecosystem to help with recovery is a consuming process.

The invasion of ERC can cause initial changes to local communities, as well as become a pathway for other invasive species to be introduced. It can possibly cause a significant impact to disrupt an ecosystem, with multiple species collapsing from inability to compete against the invader. The effects of native invader alteration of an ecosystem is warranted.

West Nile Virus

Another invasive species West Nile virus (*Flaviridae*, *Flavivirus*; WNV) is an arboviral pathogen, transmitted by mosquitoes, that was first discovered in Uganda in 1937 (Smithburn et al. 1940). From there it spread into multiple continents including Africa, Asia, Europe, North America, and South America (McIntosh et al. 1968; Hubalek and Halzouka 1999; Steele et al. 2000; Malkinson and Banet 2002; Quirin et al. 2004; Cruz et al. 2005; Mattar et al. 2005). It was discovered in New York in 1999 and then spread westward, with little understanding how the western expansion occurred (Strausbaugh et al 2001; Hayes and Gubler 2006; Kramer et al. 2019). As the virus migrated west it had undergone many mutations that allowed its transmission via new vectors, shifting from the *Cx. pipiens* as the primary vector to *Cx. tarsalis* in more rural areas (Bell et al. 2005). After the virus established and became endemic, it became the leading cause of disease from arboviruses in the United States (Lindsey et al. 2010; Curren et al. 2018; Rosenberg et al. 2018). Outbreaks typically occur during the summer and fall in temperate environments due to mosquito activity (Marra et al. 2004).

The success of the virus may be due to its reservoir hosts, in this case avian species. This type of host contains efficient levels of viremia, the concentration of virus in the blood that can be transmitted to another host, that keeps the virus in the environment. Birds as reservoirs also can move the virus along migratory pathways that allow the pathogen to be transmitted in more places. For many avian arboviruses, humans and other species are dead-end hosts because of the low levels of viremia and the pathogens inability to replicate that end with the it being killed (Farajollahi et al. 2011).

Previous studies have observed when bird reservoir hosts migrate or there is an abundance of juveniles, the mosquitoes which fed on them switch to new food sources such as humans and equine (Kilpatrick et al. 2006; Hamer et al. 2009), and these secondary or dead-end hosts can show symptoms when infected with the virus. Most human WNV infections are asymptomatic, approximately 80%, however, some cases are more serious leading to neuroinvasive disease (encephalitis, meningitis, and acute paralysis), with less than 1% resulting

in the severe cases (Mostashari 2001; Campbell et al 2002; Watson et al. 2004; Sejvar et al. 2006). These severe cases occur regularly in the Great Plains region, with an average annual incidence of greater than 1 case per 100,000, leading to a need to understand the movement of the virus (Lindsey et al. 2008).

Many of the factors which contribute to the spread of arboviruses, WNV in particular, involve human changes (anthropogenic) to the environment. For example, the development of delta regions into farming (i.e. Nile River Delta (Hayes (1989)) created a perfect breeding habitat, with slower water movement and more foliage to hide in beyond the banks of the river, which also attracted more hosts into the area. Increased foliage in an area provided habitat for birds and mosquitoes meant that people were more likely to become infected with WNV as was reported in NYC in 1999 (Brownstein et al. 2002). This increase of foliage provided the vectors with areas to rest as well as to seek their hosts, such as birds that were nesting or people that were passing. Also, regions experiencing drought often bring bird reservoirs of WNV into close proximity to people, due to limited water resources, thus increasing the chances of mosquito-borne transmission (Shaman et al. 2005; Paull et al. 2017). Drought conditions can also bring mosquitoes in closer proximity to people when finding aquatic areas suitable to lay their eggs, also increasing the chances of pathogen transmission through vectors. Other weather effects on WNV transmission include temperature and precipitation, with temp being important with effecting the rates of transmission and moisture providing suitable habitat (Reisen, 2010). The virus also exhibits a cyclical pattern, but the origin has not been identified. Two studies have found that it has a three year pattern: year one has low transmission and low human incidence, year two has high transmission and high incidence, and the third year has a reduction of transmission and incidence. This pattern does not vary by vector abundance or temperature differences (Reisen et al. 2004; Bell et al. 2005). Multiple studies have found that land use influences emerging diseases, in particular has been noted with WNV . Urbanization alters surrounding landscapes by forming heat islands. A combination of pollutants, concrete, and

asphalt absorb heat during the day and at night radiate back into the environment, raising the temperature higher than vegetative areas (Landsberg 1981). In these same areas there can be altered wind flow, change in water runoff, and pooling in drainages creating ideal habitat for vectors (Su et al. 2003). On the flip side, rural areas with higher amounts of vegetation can provide cover for the vectors and food sources for their hosts (Reisen 2010). These various landscapes also influences pathogen reservoir hosts. A study looking at the 2004 Los Angeles WNV outbreak related it back to an American crow cottonwood communal roosting site (Reisen et al. 2006). Various landscapes play essential roles in the nidus of infection.

Mosquito Vectors

Mosquitoes are in the order Diptera known as true flies, and the family Culicidae. They have a holometabolous life cycle where they go through four completely different life stages. This process begins with female mosquitoes laying eggs on water sources such as ponds, water troughs, or even bird baths with little water current. The eggs then hatch into larvae where they go through several growth stages before entering the pupa stage. After eight to ten days they emerge from the casing and become free of the aquatic environment becoming flying adults. Here the sexual differences become apparent in their feeding habits where female mosquitoes consume blood from hosts and the males consume nectar from plants. Females seek out their hosts using heat, carbon dioxide from exhaling during respiration, and octenol, a compound found in sweat. They land on locations that have easily accessible capillaries to obtain a blood meal. Using a long proboscis they probe the host to sense the flow of blood and feed straight from the capillary. This type of feeding is also known as solenophagy. The nutrients from blood meals are used for egg development to allow reproduction to continue.

Though all mosquitoes follow the same life cycle, each species differ in the various pathogens they transmit. In the United States historically Anopheles mosquitoes are known for transmitting malaria, Aedes mosquitoes typically transmit Yellow Fever and Dengue, and Culex

mosquitoes transmit West Nile Virus (WNV). Among the Culex genus there are two common vectors of WNV in the United States, *Culex pipiens* and *Culex tarsalis*, and both are found in various ecosystems across North America (Reisen and Reeves 1990; Goddard et al. 2002; Cornel et al. 2003; Reisen et al. 2008; Reisen et al. 2009). Typically, these two species feed on avian hosts, however when the birds migrate these two mosquitoes will shift their bloodmeals to mammalian species including humans and can transmit WNV.

The mosquito species *Cx. pipiens* is important to understand in both the medical and the veterinary fields. They can spread multiple pathogens including St. Louis encephalitis virus, avian malaria, and filarial worms. With WNV as the most common pathogen transmitted in the United States. Their widespread distribution also genetically and physiologically makes them difficult to identify. They are part of a large complex including *Culex pipiens pipiens*, with two forms of the species pipiens and molestus, *Culex australicus*, *Culex globocoxitus*, *Culex pipiens pallens*, and *Culex quinquefasciatus*. The two species found worldwide include *Cx pipiens pipiens* and *Cx. quinquefasciatus* which are found in urban / suburban areas in both temperate and tropical areas (Barr 1967; Farajollahi et al. 2011). They primarily feed on non-human hosts, typically avian species, however, when their preferred hosts move out of an area they will switch to mammals, such as humans (Farajollahi et al. 2011). Cases of WNV become apparent as the secondary hosts show symptoms of infection. A similar cycle is seen with the second vector found in the United States, *Cx. tarsalis*.

The second vector, *Cx. tarsalis*, became competent after the virus mutated as it moved west across the United States. According to Turell (2005) the vector competence of *Cx. tarsalis* is unsurpassed by any other North American mosquito species. They can transmit Western Equine encephalomyelitis virus, St. Louis encephalitis virus, and WNV (Reisen et al. 1995). These mosquitoes are regularly found in rural environments where they feed on avian species. Several studies have shown populations in California, Texas, and Colorado feed on birds during the spring, when avian species are nesting, but many switch to feeding on mammals after the birds

fledge and disperse (Tempelis et al. 1965, 1967, 1975, 1976, Tempelis and Washino 1967, Kent et al. 2007, Kilpatrick et al. 2006; Hamer et al. 2009). Their feeding habit makes them very competent vectors of WNV. In Colorado even with low population they were still responsible for the majority of WNV transmission among birds, and in 2003 they were responsible for 99% and 84% of the WNV risk to birds and mammals. This suggests *Cx. tarsalis* could be a sole vector among bird to bird transmission and also serve as a bridge vector to mammals (Bell et al. 2005). This species of mosquito is important to understanding the transmission of WNV.

A third minor vector found in the southern United States is the *Psorophora columbiae*, also known as the floodwater mosquito. They are considered minor vectors of WNV, but not related to outbreaks of human cases (Godsey et al 2012). This species is better well known for transmitting dog heartworm (*Dirofiliria immitis*) (Paras et al. 2014). This species is important to the transmission related to horses and should be monitored in the areas with large equine populations.

Conclusion

Understanding these three parts of disease transmission, environment, pathogen, and vector, can help researchers to identify the nidus of infection of WNV, and its ability to stay in the Great Plains region of the United States. ERC encroachment could be influencing the bird reservoir to inhabit places they were once not found. This encroachment of ERC and possible introduction of WNV increases the chances of the vectors transmitting to vulnerable hosts. Identifying the environmental effects that ERC has related to WNV and the vectors that transmit it can provide us with ways to control and decrease the impact made. Removing the threat of virus transmission may be difficult due to it being endemic, however, closing the gap in knowledge is crucial to its control. If researchers can find WNV vectors using ERC for resting, host seeking, and breeding locations, that can be important to aid in slowing the transmission processes to susceptible hosts around those areas. The research conducted will be important for

closing the gap and gaining a better understanding of both vector and pathogen relations to ERC invasion.

CHAPTER II

RESEARCH: MOSQUITO COLLECTIONS, WEST NILE VIRUS ANALYSIS AND BLOODMEAL ANALYSIS

Introduction

Eastern Redcedar (*Juniperus virginiana*) (ERC) a native species in Oklahoma, but typically found in the eastern US, was introduced into new areas in the 1930s because of its hardiness and ability to help with the soil erosion from drought conditions by creating a root system and a wind block that would help contain the soil. Later, as the ERC began expanding their range by wind and host dispersal, the lack of fires allowed the trees to quickly expand their territory (OFS, 2014). This has led to expansion in three distinct zones, in the central and western regions in Oklahoma where it previously was not nearly as abundant (Wang et al 2017, 2018).

West Nile Virus (WNV) was first recorded in 1999 in New York, in a process still not well understood. In 2001 it mutated and quickly expanded across the United States through bird reservoirs (Lanciotti et al. 1999, Kramer et al. 2019). WNV was discovered in Oklahoma in 2002, and from that point, the pathogen became endemic to the state (CDC, 2010). WNV is a viable pathogen for many hosts that can replicate in multiple bird species, that serve as reservoir hosts, and can be transmitted to new viable hosts by mosquito vectors, typically *Culex* species. Mosquitoes with WNV spread to other

reservoir hosts, or sometimes into accidental hosts, such as humans and horses/donkeys. These accidental hosts occasionally show symptoms, sometimes as extreme as neuroinvasive disease (Davis et al. 2006). These severe cases occur regularly in the Great Plains region and have led to a need to understand the vectors (Lindsey et al. 2008). Predicting WNV outbreaks requires an understanding of mosquito ecology, specifically for *Culex tarsalis* and *Cx. pipiens*, the main vectors in the southern regions of the United States.

The *Cx. tarsalis* mainly inhabits rural areas and have strong flight abilities, making them viable vectors for this region (Reisen, 1995). Previous studies have shown this species inhabiting ERC to seek their hosts (O'Brien & Reiskind, 2013). More recently, results from Cote's research indicate that different *Culex* species prefer different types of ERC canopies. *Cx. pipiens* were found to be more abundant in closed ERCs, where the tree canopy has very little openings. in central Oklahoma, whereas *Cx. tarsalis* were found in both open, spread canopy cover, and closed ERCs in western Oklahoma. Additionally, 8 of the 9 positive WNV pools of *Cx. pipiens* and *Cx. tarsalis* were collected in ERC habitat. These two studies have focused attention on whether the ERC invading Oklahoma may be contributing to WNV infections in the region. Another study with ticks has also seen ERC being a contributing factor (Noden & Dubie, 2017).

Given these aspects of ERC expansion and the need to focus on *Culex* sp. in the region, the aim of our study was to evaluate how mosquito populations are impacted by varying concentrations of ERC in different expansion areas in Oklahoma. To focus on these relationships, we tested the following hypotheses: 1) The abundance of mosquito communities are directly impacted by increasing concentrations of ERC in different regions of the state. 2) WNV-infected mosquitoes will be more likely to be collected in

ERC than in grassland. We tried to evaluate host preference of blood-fed mosquitoes using ERC which would provide an understanding of host-mosquito interactions in ERC and how they could be using the invasive species to seek hosts. To do this, we compared four methods for recovering blood-fed mosquitoes in ERC that had not been used in the Great Plains region. These included comparing CDC Light traps baited with CO_2, which are used to collect host seeking females, as well as, four types of resting traps: CDC gravid traps, 32 gallon bucket traps, wire-frame shelter traps, and fiber pot traps, which used to collect resting blood-fed mosquitoes.

Materials and Methods

Study Locations

Sites were selected based on previous research that identified three expansion zones in central and western Oklahoma (Wang et al, 2017 & 2018). From the expansion zones identified, we identified sites of differing ERC concentrations in seven counties through coordination with county extension personnel and landowners (Table 1). Because of the nature of the study, study sites were chosen based on accessibility and producer permission. As such, our sites ended up grouping according to different concentration of ERC at each site: Grassland, 0.1-1.0% ERC coverage, 1.1-20%, 21-40%, 41-60%). All sites had livestock, cattle at six of the sites and horses at one site that grazed in or around the sampling areas. Mosquito collections occurred in 4 different ERC expansion regions to ensure that relationships observed between mosquito abundance and ERC levels was the same throughout the state. In total, there were 32 sampling sites within seven counties. Zone one included Stillwater OSU Cross-timbers Research Facility (Payne County) and Ringer's farm in Mulhall (Logan County). Zone two

consisted of two sites: one east of Watonga (Blaine County) and the other near the Cimarron River, west of Hennessey (Kingfisher County). Zone three consisted of one site: south of Binger (Caddo County). Zone four included a site east of Joy (Murray County), and one site north of Lindsay (McClain County). Each collection site was spaced 200 meters apart to increase variability and prevent over sampling an area. The county sites varied on number of sampling areas depending on land available and cedar trees in the area. Payne, Blaine, and Caddo had six sites. McClain and Murray had four sites, and Logan and Kingfisher had three sites.

Table 1. The county, zone, and site numbers with latitude and longitude locations and the number of each style trap used. The Z integer stands for the zone number assigned and the S integer stands for the site in the Site column.

County	Site	Latitude	Longitude	ERC Density	No. CDC Traps	No. Gravid Traps	No. Fiber Pot Traps	No. Bag and Wire Traps	No. Bucket Traps
Payne	Z1S1	36.0699619	-97.192475	0.5978	2	1	2	1	1
	Z1S2	36.0679321	-97.1920993	8.4044	2	1	2	1	1
	Z1S3	36.0598348	-97.1829629	44.4957	2	1	2	1	1
	Z1S4	36.0601346	-97.1788497	59.4972	2	1	2	1	1
	Z1S5	36.0571503	-97.1797959	11.0228	2	1	2	1	1
	Z1S6	36.0571503	-97.1797959	27.7213	2	1	2	1	1
Logan	Z1S7	36.0250097	-97.45554	0	2	1	0	0	0
	Z1S8	36.0250752	-97.4546713	0.4769	2	1	0	0	0
	Z1S9	36.0235297	-97.4524457	1.041	2	1	0	0	0
Blaine	Z2S1	35.8473531	-98.3166339	0	2	1	2	1	1
	Z2S2	35.8490928	-98.3169674	21.674	2	1	2	1	1
	Z2S3	35.8507492	-98.3165272	15.417	2	1	2	1	1
	Z2S4	35.8503312	-98.3150722	7.8154	2	1	2	1	1
	Z2S5	35.849669	-98.3141579	9.6948	2	1	2	1	1
	Z2S6	35.8475388	-98.3151703	0.4636	2	1	2	1	1
Kingfisher	Z2S7	36.113711	-98.1647118	1.5802	2	1	0	0	0
	Z2S8	36.1100348	-98.1631033	22.7813	2	1	0	0	0
	Z2S9	36.1100348	-98.1631033	2.7734	2	1	0	0	0
Caddo	Z3S1	35.2738958	-98.3441269	0.2202	2	1	2	1	1
	Z3S2	35.2730055	-98.347176	3.3677	2	1	2	1	1
	Z3S3	35.2751164	-98.3475734	25.2928	2	1	2	1	1
	Z3S4	35.2768546	-98.3481158	18.6951	2	1	2	1	1
	Z3S5	35.2769792	-98.3465536	18.5129	2	1	2	1	1
	Z3S6	35.2756144	-98.3456715	26.036	2	1	2	1	1
Murray	Z4S1	34.5900998	-97.0966103	0	2	1	2	1	1
	Z4S2	34.5904611	-97.1045776	0.7862	2	1	2	1	1
	Z4S3	34.5904611	-97.1045776	10.6757	2	1	2	1	1
	Z4S4	34.5904611	-97.1045776	20.3972	2	1	2	1	1
McClain	Z4S5	34.9503546	-97.5888787	0.7731	2	1	2	1	1
	Z4S6	34.9503546	-97.5888787	15.5946	2	1	2	1	1
	Z4S7	34.945605	-97.5875741	22.5835	2	1	2	1	1
	Z4S8	34.945605	-97.5875741	0.6573	2	1	2	1	1

Each site had a mix of grassland and ERC. Zones one and four are both located in the cross-timbers region of Oklahoma and had a higher mix of woody vegetation mixed with the ERC. Individual sites from each sampling area were analyzed by using Google

Earth aerial imagery, to identify ERC areas, and visually checked for digital accuracy. All sampling locations had at least one grassland site as the control site, one low-moderate (1-18%) ERC density site, and one higher ERC (19-60%) density site. The selected sites had pixel statistical analysis to identify the percentage of ERC in the area.

Trapping Protocol

Bi-weekly collections at the sites began 8 June 2020 and ran through 17 August 2020 with two zones visited each week for one night of sampling. Tri-weekly collections began 26 September 2020. Collections in zones one and two rotated with zones three and four on opposite weeks. Each county was sampled for one night, where CDC and Gravid traps were set by 1500 and all traps were picked up the following day by 1200. This ensured little disturbance occurred before crepuscular and nocturnal species became active. The resting traps were set prior to first week of sampling from 1 June through 4 June at the Payne, Watonga, Binger, Joy, and Lindsay sites. Multiple trapping techniques were used to sample for female mosquitoes. These included CDC Light Traps (Bioquip, Rancho Dominguez, CA) (Appendix Figure 1), without lights and baited with two pounds of dry ice, to attract host-seeking females. CDC Gravid traps (John W. Hock Company, Gainesville, FL) (Appendix Fig. 2), set-up at the time of the CDC light traps, using hay-infusion water, were used to attract egg laying females. Three types of resting traps: fiber planting pots (Greenhouse Megastore) (Burket-Cadena et al., 2008) (Appendix Fig. 3), that were nine inches by nine inches, bag and wire traps (Burket-Cadena et al, 2008) (Appendix Fig. 4) with wire (Lowes) cut two meters in length and then wrapped around on itself secured with four zip ties to make one meter diameter openings with 32 gallon trash bags tied around the wire, and 32 gallon black trash cans (Lowes) (Burket-Cadena

et al., 2011) (Appendix Fig. 5) used to collect female mosquitoes resting while digesting recent blood meals. Resting traps were left at the sites for the duration of the study.

Sites varied by traps used. For all sites, two CDC light traps and one gravid trap were used in each ERC concentration and grass sampling site. The resting traps, two fiber pots, one bag and wire, and one bucket trap, were placed at the sampling sites in Payne, Blaine, Caddo, McClain, and Murray counties. Logan and Kingfisher counties did not have resting traps placed at them. The sites with all traps had traps in each density coverage range.

CDC traps in ERC locations were hung one to two meters off the ground midway on branch toward trunk of tree. The gravid traps were place inside the foliage close to the base of ERC. Resting traps were fastened to lower branches with openings facing toward the outside. Two fiber pot traps were at each site, one was placed approximately one meter off the ground and the other was placed on the ground. At the time of collection, all resting traps were sampled using InsectaZooka Field Aspirator (Bioquip, Rancho Dominguez, CA), aspirating all sides of the trap as well as any foliage surrounding. Only one extension was used for InsectaZooka, allowing easy movement inside the trap to reach all surface angles. After aspiration, resting traps were moved to different ERC within the same site and reset at random for the following visit.

Sample Sorting and Identification

Mosquitoes collected from either CDC or Gravid traps were secured in collection nets and labeled with date, zone, site, and trap type. Mosquitoes collected from resting traps were capped off in the InsectaZooka collection containers, a clear propylene cup with mesh aluminum screen inlayed into the bottom (Bioquip, Rancho Dominguez, CA),

and labeled with same tag information as the previous style traps. Collected specimens were placed in a Whynter Portable Freezer (85 quart, Whynter, Brea, CA), for quick euthanasia and storage during transportation, and later stored at a -20^{o}C. Identifications were completed at a later time using Darsie and Ward (2005). Upon identification, specimens were placed into snap cap vials (7-dram, Fisher Scientific, Hampton, NH) and labeled with date, zone, site, trap, genus and species then returned to -20°C for later analysis. Species identified for further analysis included both *Culex pipiens* and *Cx. tarsalis* mosquitoes, arranged by date and site collected for WNV analysis. Due to southern Oklahoma being a hybrid zone, *Culex pipiens* and *Culex quinquefasciatus* were identified as *Culex pipiens* L. complex (Harbach 2012). All blood-fed mosquitoes, from any species, were also separated individually by date and site collected for bloodmeal analysis and were stored at -20°C.

West Nile Virus Analysis

West Nile Virus analysis was conducted on the previously collected *Culex pipiens* and *Culex tarsalis* mosquitoes starting 27 October 2021 and completed 20 November 2021. The mosquitoes were initially processed by pooling into groups of up to 25 individuals by collection date, site, trap type and species. If mosquitoes were blood-fed, only head and legs were used, thorax and abdomens were saved for bloodmeal analysis. A master mix of phosphate-buffered saline (PBS) and 2x lysis buffer were combined into a 50:50 mixture (Applied Biosystems, Foster City, CA). All tubes were labeled for extractions beforehand. 200 µL of the master mix was added to each pool (300 µL for pools made up of 8 mosquitoes or more) as well as two 3.2 mm stainless steel beads (Biospec Products, Bartlesville, OK) to special vials (Biospec) developed for beating

with steel beads. Mosquitoes were then added to appropriate tubes. Tubes were then placed into the mixer mill (Biospec, Bartlesville, OK) for 4 min, 2 rounds of 2 minutes with tops checked in between for loosening to keep from losing buffers. Samples were centrifuged at 6,000 rpm for 4 min and supernatants were placed into new labelled vials. They were then stored in -80^{o}C freezers for preservation.

Once extractions were completed mosquito samples were transported on ice for total RNA extraction using the QIAmp Viral RNA Mini Kit (250) (Qiagen) and following the manufacturers protocol. Samples were again stored in -80^{o}C. Real-time RT-PCR was performed on RNA extracted from the mosquitoes using a combination of a QuantiTect Probe RT-PCR Kit (Qiagen), 25 pmol of WNV primers (Lanciotti et al. 2000), 3.25 pmol of the probe, and 10 µL of the RNA extracted from the mosquitoes for a total reaction volume of 25µL. Real-time PCR amplification of the reaction mix was performed on Rotor-Gene 6000. A single cycle of 50^{o}C for 30min (reverse transcription) and 95^{o}C for 15 min (hot start), followed by 40 cycles of 94^{o}C for 30s, 55^{o}C for 1min, and 68^{o}C for 1 min. Result reports were created after cycles were completed. Positive controls were graciously provided by Dr. Gabriel Hamer (Texas A & M).

Bloodmeal Analysis

All species of blood-fed mosquitoes were processed for analysis. Mosquitoes were processed separately and were placed into separate tubes and organized by collection date, site, trap type, and species. A master mix was created of a 50:50 of phosphate-buffered saline (PBS) and 2x lysis buffer (Applied Biosystems, Foster City, CA). Special containers from Biospec were used for bead beating process. 200µL of master mix was added to each individual tube as well as 20µL Protease K, to help extract

DNA. The mosquitoes were then added into corresponding tube and placed into incubator (Fisher Scientific) for 30 min to rehydrate specimens. Two 3.2mm stainless steel beads (Biospec Products, Bartlesville, OK) were added to the tubes and placed into the mixer mill (Biospec, Bartlesville, OK) for 4 min, 2 rounds of 2 minutes, with tops checked in between for loosening to keep from losing buffers. Samples were then centrifuged at 6,000 rpm for 4 min and supernatants were placed into new labelled vials. Samples were stored in -20^{o}C until the DNA purification process began.

The initiated blood meals were identified by polymerase chain reaction (PCR) amplification and DNA sequencing of a fragment of either the vertebrate mitochondrial cytochrome c oxidase 1 (COI) or cytochrome b (cytb) genes (Kent et al. 2009; Thiemann et al. 2011). However, after trying all three assays with a wider variety of different primer sets, we were not able to amplify anything from the samples processed.

Statistics

This study focused on mosquito communities found in varying density of ERC. Collections only occurred during summer, so to test the hypothesis that ERC densities affect mosquito abundance, we analyzed the influence of varying levels of ERC on the mean abundance of mosquitoes collected at each site. The influence of ERC concentration on the mean abundance of important mosquito vector species (*Ae. albopictus, An. quadrimaculatus, Cx. pipiens, Cx. tarsalis, and Ps. columbiae*). Statistical analysis was completed using SAS JMP Pro 15 (SAS Institute, Cary, NC, USA). ERC density at each site was calculated using Python from Google Earth Pro aerial images of each site. These were cropped down to fit sites and then labeled using Blender Image Editor where ERC trees were painted over in white

(Appendix Fig. 6). All non-white painted pixels were changed to black and images were saved into grayscale. A percentage of white to total pixels was calculated providing the numerical value for ERC density for each site sampled. Patterns in the data were analyzed using ANOVA. Initial analysis included 5 categories of sites based on ERC concentration (0% ERC cover, 0.1-1.0%, 1.1-20%, 21-40%, 41-60%). To accommodate differences in numbers due to trap failures, total mosquitoes collected by site were divided by number of collection nights at each site. This value, was Log+1 transformed for normality and homogeneity of variance and one-way ANOVA analyses were used to compare the mean abundance of each species between the five habitat types . Because of the challenges in funding and limited collection opportunities during the main period of *Cx. tarsalis* activity, we evaluated *Cx. tarsalis* in the country where the majority were collected (Blaine county) by one-way ANOVA analysis using abundance collected in grass vs ERC. Next, linear regression analysis was used to plot log+1 transformed mean abundance for species by site against percentage of surrounding ERC was used. Finally, a mixed model analysis (SAS JMP 15) with log+1-transformed mean abundance of mosquitoes by species as the dependent variable and collection round and ERC percentage as fixed variables. To ensure that the relationship was common throughout all ERC encroached regions, sites was used as a random effect to account for non-independence of sites for any given collection period. For each model, residual plots were within the correct residual quantile plots.

Results

Collection Results

Between June and September 2020, 32 sampling sites were established in seven counties involving differing concentrations of ERC in zones where ERC is expanding (Fig. 1 and 2). Over the course of the study, trap failures did occur. CDC light trap failure occurred 4 times (June 23 – Z1S2 – rodent chewed wire; June 30 – Z4S4 – cow disturbance; July 13 – Z3S4 – rodent chewed wire; July 30 – Z4S1 – storm damage) and gravid traps failed 4 times (Aug 6 – Z2S8; Aug 17 – Z1S8; Aug 21 – Z2S9, Sept 11 – Z2S8). Buckets went missing twice – June 11 – Z2S1; Sept 4 – Z1S5 (controlled burn).

A total of 5,791 female mosquitoes were collected involving a total of 160 trap nights (every trap (CDC light traps)/night/week x 5 visits) (Table 2). The most mosquitoes were collected in Zone 2 (n=2,027 (35.0%) followed by zone 4 (n=1, 934 (33.4%), zone 1 (n=1,164 (20.0%) and zone 3 (n=415 (7.2%) (Table 2). The majority of mosquitoes (n=5,558) were collected using CO_2-baited CDC light traps (95.97% of the total mosquitoes) followed by gravid traps (n=131), bucket resting traps (n=86), bag and wire (n=16) and fiber pot (n=0).

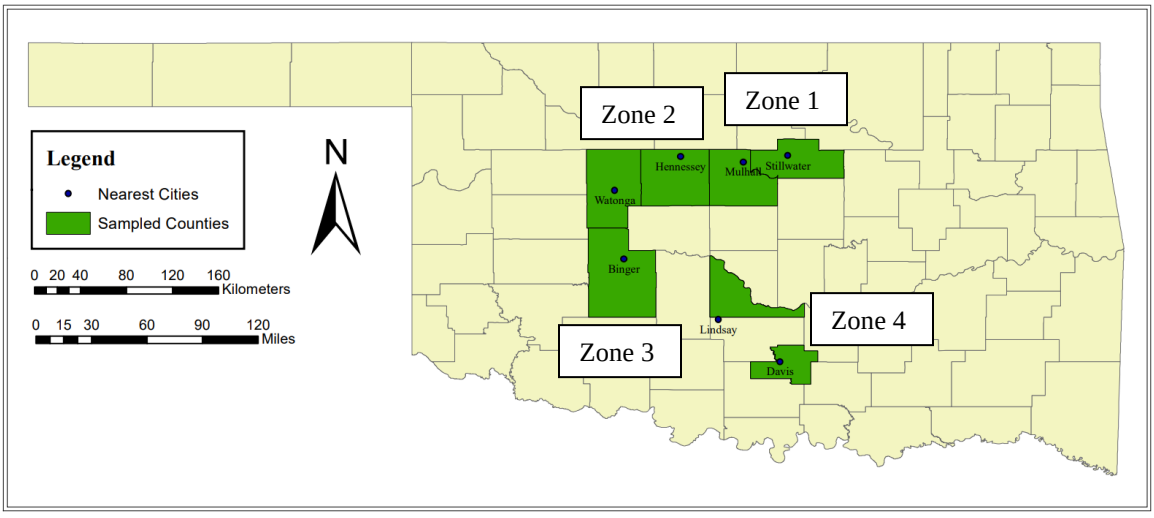

Figure 1. Map of Oklahoma showing the counties sampled in and the nearest city to the sites.

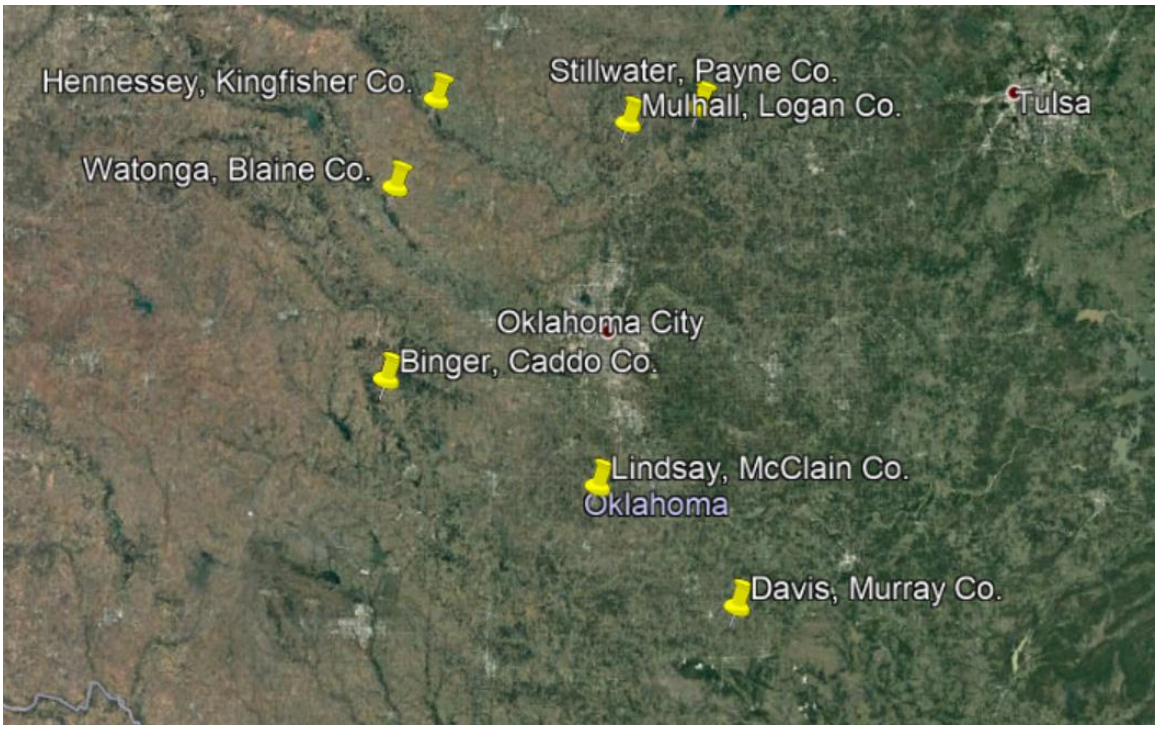

Figure 2. Aerial photograph with site locations labeled. Taken from Google Earth Pro.

Table 2. Comparison of species collected in each zone, site, and trap type. There were no mosquitoes collected from the fiber pot traps.

Mosquitoes	Location								Trap Type				
	Zone 1		Zone 2		Zone 3	Zone 4							
Species	Stillwater	Mulhall	Watonga	Okeene	Binger	Joy	Lindsay	Total	CDC Light	Gravid	Bucket	Wire	Total
Ae. albopictus	15	3	7	0	17	1	3	**46**	32	14	0	0	**46**
Ae. canadensis	0	0	0	4	0	0	2	**6**	6	0	0	0	**6**
Ae. epactius	0	0	0	0	0	2	0	**2**	2	0	0	0	**2**
Ae. sollicitans	15	45	32	7	1	24	25	**149**	149	0	0	0	**149**
Ae. vexans	4	2	7	18	10	44	36	**121**	121	0	0	0	**121**
Ae. zoosophus	7		8	1	25	4	13	**58**	34	23	0	1	**58**
An. crucians	0	0	0	0	0	2	0	**2**	2	0	0	0	**2**
An. punctipennis	3	7	176	0	5	2	9	**202**	154	7	36	5	**202**
An. quadrimaculatus	1	37	35	9	2	19	11	**114**	105	7	2	0	**114**
Cx. coronator	1	1	11	1	19	4	0	**37**	20	10	7	0	**37**
Cx. erraticus	22	574	401	44	235	760	541	**2577**	2545	23	8	1	**2577**
Cx. nigripalpus	2	4	18	0	3	74	6	**107**	107	0	0	0	**107**
Cx. pipiens	2	5	60	6	5	22	6	**106**	97	9	0	0	**106**
Cx. perturbans	0	0	11	0	0	0	0	**11**	11	0	0	0	**11**
Cx. salinarius	0	0	0	0	0	0	3	**3**	3	0	0	0	**3**
Cx. stigmatosoma	0	0	1	0	0	0	0	**1**	1	0	0	0	**1**
Cx. tarsalis	0	22	74	86	83	40	64	**369**	368	1	0	0	**369**
Ps. ciliata	2	19	1	2	0	3	4	**31**	32	0	0	0	**32**
Ps. columbiae	144	196	590	395	36	99	76	**1536**	1531	5	0	0	**1536**
Ps. cyanescens	18	33	43	65	2	14	61	**236**	234	2	0	0	**236**

Table 3. Total numbers of important mosquito vectors collected by trap site compared to ERC percent density.

City	Trap	ERC %	*Ae. albopictus*	*An. quadrimaculatus*	*Cx. pipiens*	*Cx. tarsalis*	*Ps. columbiae*
Stillwater	Z1S1	0.5978	0	0	0	0	120
	Z1S2	8.4044	2	1	0	0	10
	Z1S3	44.4957	6	0	0	0	1
	Z1S4	59.4972	3	0	2	0	0
	Z1S5	11.0228	2	0	0	0	3
	Z1S6	27.7213	2	0	0	0	10
Mulhall	Z1S7	0	0	27	3	9	143
	Z1S8	0.4769	0	0	0	12	7
	Z1S9	1.041	3	10	2	1	46
Watonga	Z2S1	0	1	4	9	7	184
	Z2S2	21.674	3	2	2	16	16
	Z2S3	15.417	1	13	23	15	121
	Z2S4	7.8154	1	4	9	10	105
	Z2S5	9.6948	1	9	10	11	71
	Z2S6	0.4636	0	3	7	15	93
Okeene	Z2S7	1.5802	0	9	6	29	213
	Z2S8	22.7813	0	0	0	20	97
	Z2S9	2.7734	0	0	0	37	85
Binger	Z3S1	0.2202	0	1	0	8	14
	Z3S2	3.3677	2	0	0	11	7
	Z3S3	25.2928	3	0	1	9	5
	Z3S4	18.6951	4	0	0	7	0
	Z3S5	18.5129	4	0	4	12	3
	Z3S6	26.036	4	0	0	36	7
Joy	Z4S1	0	0	2	1	14	55
	Z4S2	0.7862	0	8	6	10	18
	Z4S3	10.6757	0	6	3	2	22
	Z4S4	20.3972	1	3	12	14	4
Lindsay	Z4S5	0.7731	2	1	2	9	35
	Z4S6	15.5946	0	7	1	13	3
	Z4S7	22.5835	1	3	0	29	10
	Z4S8	0.6573	0	0	3	13	28

Of the medically-important species of mosquitoes collected, the most common species collected was *Psorophora columbiae* (n=1,532) followed by the two major vectors, *Culex pipiens* (n=106) and *Cx. tarsalis* (n=369) (Table 3). The least number collected were *Aedes albopictus* (n=46).

The mean abundance of *Ae. albopictus* (F ratio=3.737, df=4, P=0.0152) and *Ps. columbiae* (F ratio=4.6480, df=4, P=0.0055) were strongly related to increasing (*Ae. albopictus*) (Fig 3A) and decreasing (*Ps. columbiae*) (Fig 3B) concentration of ERC. For *Ae. albopictus*, the highest density of ERC (41-60%) were significantly higher than in the grassland sites and lowest density of ERC (0.1-1.0%) (Fig 3A). Conversely, for *Ps. columbiae*, grassland sites were significantly higher than any ERC site above 1.0% encroachment (Fig 3B). No relationships were identified for other species except when

comparing grass vs ERC in Blaine county for *Cx. tarsalis* (F ratio=7.716, df=1, P=0.0499).

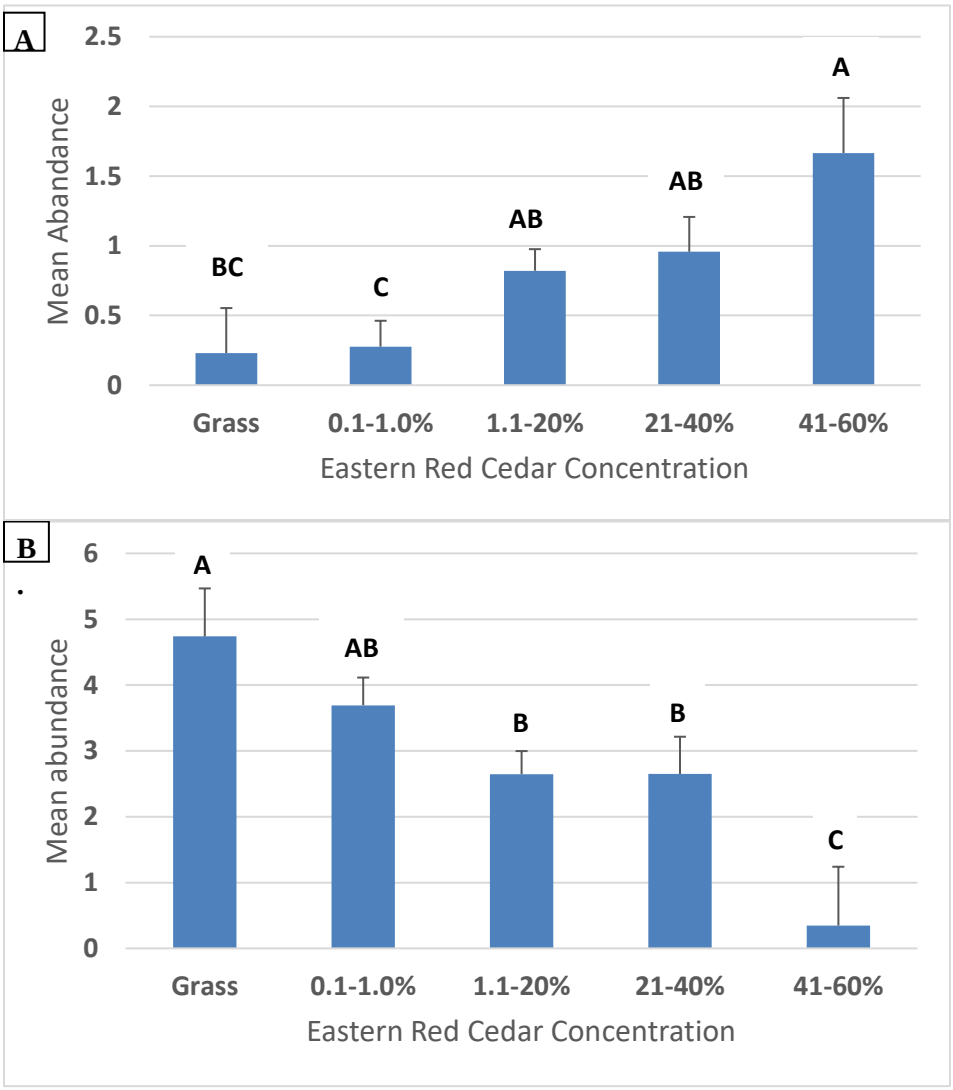

Figure 3. Log +1 Mean Abundance (+SE) (A) *Ae. albopictus* and (B) *Ps. columbiae* in differing concentrations of ERC in Oklahoma.

Linear regression analysis followed what was identified with the ANOVA analysis. Mean abundance of *Ae. albopictus* increased with increasing percentage of surrounding ERC (R^2=0.36, F ratio=17.34, df=31, P=0.0002) (Figure 4A) while *Ps.*

columbiae decreased (R^2=0.41, F ratio=13.61, df=31, P<0.0001) (Figure 4B).

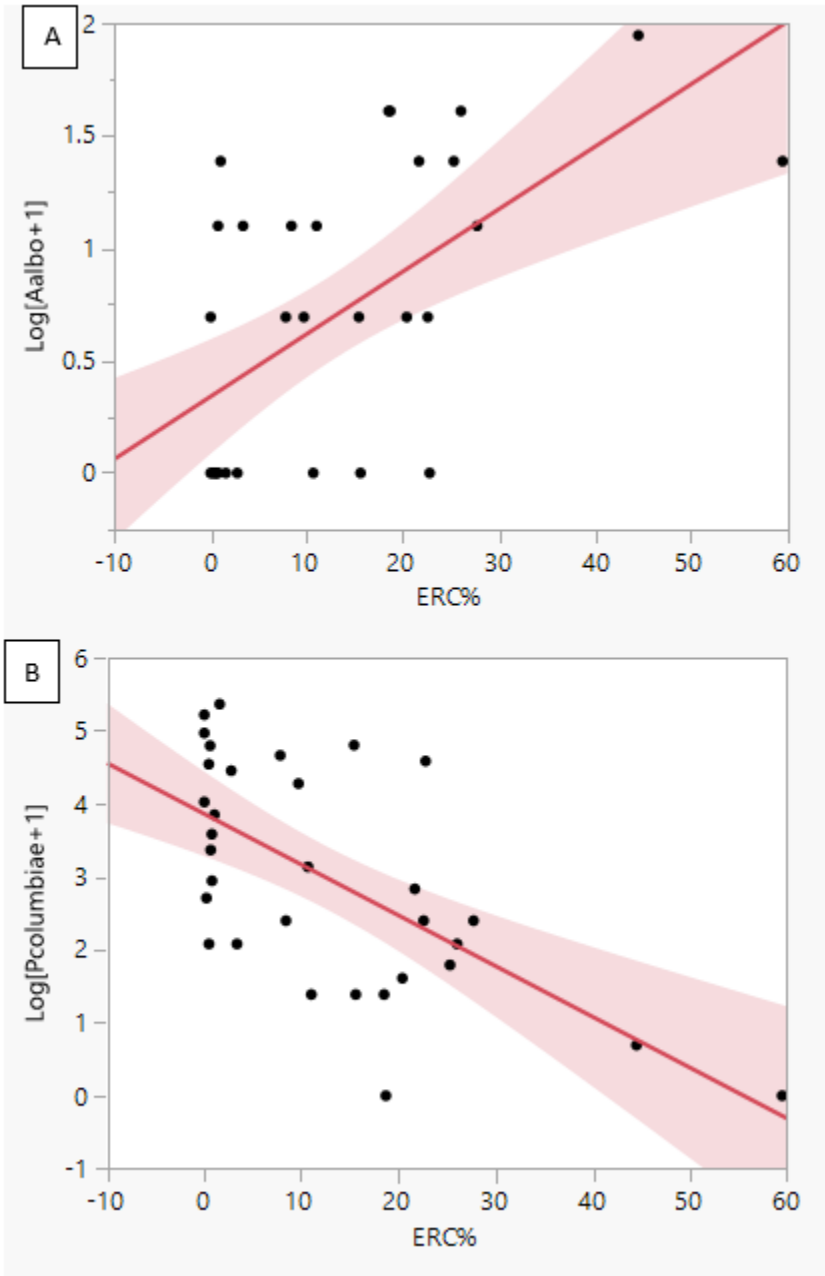

Figure 4. Linear relationships between mean abundance of *Ae. albopictus* (A) and *Ps. columbiae* (B) by increasing percentage of ERC surrounding each site.

Using mixed methods analysis, mean abundance of *Ae. albopictus* was significantly related to increasing ERC percentage ($F_{1,35}$=17.06; P=0.0002) and sampling period (($F_{5,155}$=5.83; P<0.0001) (Table 4). Additionally, mean abundance of *Ps. columbiae* was significantly related to decreasing ERC percentage ($F_{1,43}$=18.00; P=0.0001) and sampling period ($F_{5,154}$=19.29; P<0.0001) (Table 4). Mean abundance of *Cx. tarsalis* was significantly related to sampling period ($F_{5,155}$=43.09; P<0.0001) but not ERC percentage ($F_{1,41}$=2.81; P=0.1014) (Table 4). No significance was found between ERC percentage and mean abundance for *Cx. tarsalis* or *Cx. pipiens*.

Table 4. General linear mixed models testing the effects of eastern red cedar concentration and sampling period on mean abundance of *Ae. albopictus*, *Ps. columbiae*, and *Cx. tarsalis* collected.

	Ae. albopictus			*Ps. columbiae*			*Cx. tarsalis*		
Variable	F	df	P	F	df	P	F	df	P
Sampling period	5.83	5,155	0.0001	19.288	5,154	<0.0001	43.09	5,155	0.0001
ERC percentage	17.06	1,35	0.0002	18.003	1,43	0.0001	2.81	1,41	0.1014

West Nile Virus Results

A total of 111 pools were tested for WNV, 44 pools for *Cx. pipiens* and 67 pools for *Cx. tarsalis*. Only one pool of *Cx. tarsalis* tested positive (Figure 5). The pool was made up of 18 *Cx. tarsalis* mosquitoes collected from a CDC light trap in Zone 3 Site 6, from Binger (Caddo county), OK, on 19 September 2020. Zone 3 Site 6 has a 26.04% coverage of ERC, one of the higher density sites sampled in that zone.

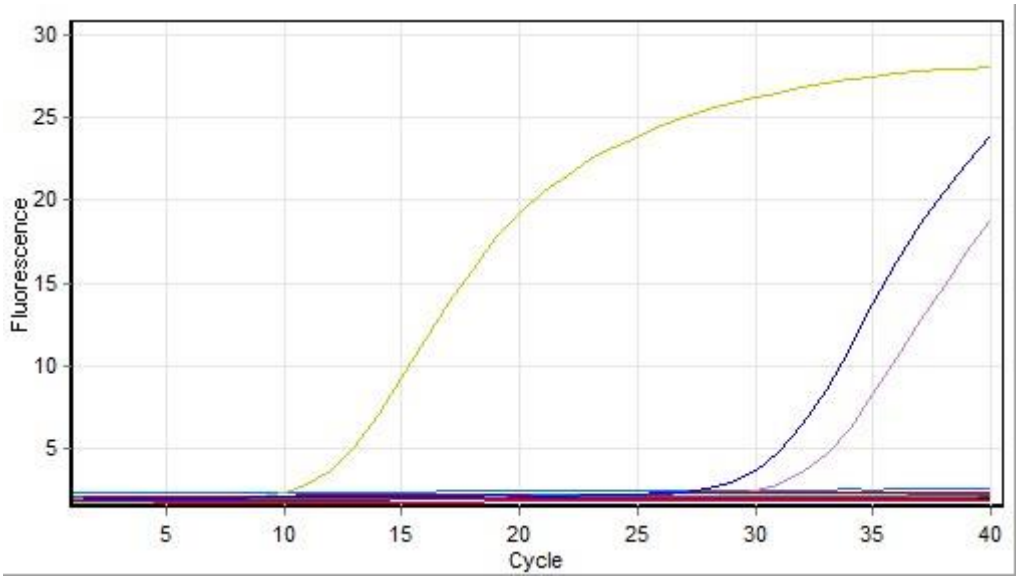

Fig 5. qPCR results from WNV analysis for positive pool. WNV positive pool 51 confirmed on cycle 28 (purple). Positive controls WNV GBlock appear on cycle 8 (yellow) and the WNV RNA appears on cycle 26 (black).

Bloodmeal Results

Of the 94 blood-fed mosquitoes collected, the majority (n=77 – 82.8%) were collected using CO_2-baited CDC light traps. Of the 17 remaining blood-fed mosquitoes collected, 14 were collected in bucket traps followed by the wire/bag trap (n=2) and gravid trap (n=1). The majority of blood-feds collected (n= 49 (52.1%)) were *Ps. columbiae*, followed by *An. punctipennis* (n=14), *Cx. erraticus* (n=10), *Ae. sollicitans* (n=8), *An. quadrimaculatus* (n=5), *Ps. cyanescens* (n=4), *Ae. vexans* (n=2), *Ae. albopictus* and *Cx. tarsalis* (n=1). Of the 79 gravid mosquitoes collected, the majority (n=54 (68.4%)) were collected by CDC light traps, followed by gravid traps (n=25 (31.6%)) followed by bucket traps (n=8) and wire/bag (n=1). Gravid mosquitoes comprised a diverse number of different species: *Ae. albopictus* (n=3), *Ps. columbiae*

(n=4), *Cx. coronator* (n=1), *Cx. erraticus* (n=33), *Cx. pipiens* (n=3), *An. punctipennis* (n=9), *Cx. tarsalis* (n=20), *Ae. vexans* (n=3), and *Ae. zoosophus* (n=3).

PCR analysis of bloodmeals was not completed due to DNA degradation from improper storage of specimens. They were stored in -20^{o}C and should have been stored in -80^{o}C to preserve the samples.

Discussion

Based on mosquito collections in three ERC expansion areas in central and western Oklahoma, we found that differing concentrations of ERC have an effect on mosquito abundance. Abundance of *Aedes albopictus* was directly related to higher concentrations of ERC while *Ps. columbiae* abundance was inversely related to increasing ERC concentration. The only impact of ERC on *Cx. tarsalis* occurred in western Oklahoma in Blaine County with more *Cx. tarsalis* collected in ERC than the grassland control site. Secondly, the only WNV-infected pool of mosquitoes detected were *Cx. tarsalis* collected in ERC in September. CDC light traps were more successful at collecting blood-fed and gravid mosquitoes than other methods used, the third hypothesis was not addressed due to DNA degradation in freezer storage (Reeves et al. 2016). Finally, among the resting traps tested, 32 gallon black bucket was most successful for collecting blood-fed mosquitoes than the bag/wire trap and fiber pot traps.

Previous studies have identified that ERC impacts mosquito communities, *Culex pipiens* and *Cx. tarsalis*, in particular, with higher abundance of mosquitoes collected in ERC compared with grassland sites (O'Brien & Reiskind 2013; Noden and Cote, unpublished data). This study has further defined those relationships and identified that abundance of adult host-seeking *Ae. albopictus* and *Ps. columbiae* are impacted by

differing concentrations of ERC. Although not a primary vector, *Ae. albopictus* is considered a bridge vectors for WNV (Rochlin et al. 2019) and a competent vector for multiple human (Yellow Fever, Dengue, Chikungunya, and Zika) and animal pathogens (Eastern Equine Encephalitis, Western Equine Encephalitis, and dog heartworm (*Dirofilaria immitis*)) (Mitchell et al. 1987; Miller and Ballinger 1988; Mitchell and Miller 1990; Scott et al. 1990; Beaman and Turell 1991; Mitchell 1991; Licitra et al. 2010, Paras et al. 2014). Identifying a higher abundance of *Ps. columbiae* in grassland or areas with small ERC densities is also important. Primarily considered a nuisance mosquito for cattle and horses (Kuntz et al. 1982), *Ps. columbiae* is considered a possible minor vector of WNV, but is not considered an issue for human outbreaks (Godsey et al. 2012). The species also has shown potential to transmit Venezuelan Equine Encephalitis Virus, Rift Valley Fever Virus, as well as, dog heartworm (Moncayo et al. 2008; Paras et al. 2014; Turrell et al. 2015). This species is also known to inhabit flood waters with the average rainfall in the four zones ranging between 3.26 inches and 4.325 inches for the months of June and September.

While *Cx. tarsalis*, the main vector for WNV in western Oklahoma (Noden et al. 2015) was only found more likely to be present in ERC rather than grassland, the fact that most of the collection period for this study was outside the peak period for the species, between September and November (Cote, unpublished data), activity in the region indicates that more focus needs to be made on this important species in the future. It is important to note that the only WNV-infected mosquitoes collected were *Cx. tarsalis* in ERC from the last collection time (mid-September) at one of the sites in SW Oklahoma. The potential association of increasing abundance of an important mosquito vector (*Ae. albopictus*) with increasing concentrations of ERC in addition to the continued

association of WNV-infected *Cx. tarsalis* with ERC needs to be studied further, particularly as it applies to increasing levels of ERC in urban areas. These areas carry more risk with a higher population of susceptible hosts.

While it was not possible to identify the hosts from blood-fed mosquitoes, this study provided important information regarding the best traps to use for collecting blood-fed and gravid mosquitoes. CDC light traps are the best method for collecting a high diversity of mosquito species. While this observation has been made by others (Reiskind et al. 2017), the current study also demonstrated the usefulness of CDC light traps in the collection of the majority of blood-fed and gravid mosquitoes. In our study design, gravid traps or any of the three resting traps did not have much success. This is most likely due to the resting traps only being visited one morning every two weeks and gravid traps being set up and taken down within 24 hours which favored the mosquitoes that were host-seeking rather than resting or gravid. If traps were left up and visited multiple days consecutively, a higher success rates for the gravid and resting traps may have been achieved (Burket-Cadena et al., 2008; 2011).

No study is without limitation, but it was worked within the challenges that occurred. One of the main challenges was to carry out a full-season collection study within the confines of national and university COVID-19 protocols as well as a funding cut at the end of July. The COVID protocols limited how early we could establish collection sites and limited the number of interacting people which changed how field work could be performed and overnight accommodation. The ending of funding at the end of July meant that limited alternative funding sources were required to accommodate longer sampling periods. These two limitations were further constrained when the sampling times were shifted from a bi-weekly rotation to a tri-weekly rotation due to

school schedule, thus decreasing the number of visits to all sites for the final month and half of sampling just as *Culex* sp. numbers were peaking. Finally, due to limited funding, we used old gravid traps which failed often due to motor failure or old batteries. Given all these challenges, however, we collected a representative number of mosquitoes throughout the study period which provided relationships between mosquito communities and differing densities of ERC that can be followed up with in the future.

Conclusion

In summary, this study provided much needed data that will help us to identify the gaps on the ecology of important mosquito vectors and give us a better idea of the nidus of infection in the Great Plains. This study identified that some important mosquito species which transmit pathogens to humans and animals may vary with changing ERC densities. Although we were not able to determine host preferences, we did identify CDC Light traps baited with CO_2 were best for collecting blood-fed mosquitoes and *Ps. columbia*e were most likely to be collected in ERC-invaded areas. By better understanding the ecology of the mosquitoes of vector borne disease transmission in the southern Great Plains, we may be able to identify ways to predict and prevent possible pathogen outbreaks. If specific mosquito vector species utilize certain identifiable features on the landscape (ERC), we may be able to predict areas of higher probability of finding infected mosquitoes and it may provide an important means to control the presence of specific vector species. In the future, more detailed study designs will focus on the entire mosquito season over the course of a couple years in addition to focusing on vector-host interactions with varying densities of ERC.

CHAPTER III

INFORMATION FOR PRODUCERS

Ever since West Nile Virus (WNV) entered the United States in 1999 it has been a concern for the public. It has become the highest transmitted arthropod borne virus in the United States. The primary hosts, also considered reservoir hosts, are birds, and typically the virus rarely affects them. However, when the virus is introduced to the secondary host, or also known as accidental hosts, such as humans and equine, symptoms can be more severe. The range of effects can be anything from cold-like symptoms to the more severe cases such as neuroinvasive disease. These severe symptoms has a higher incidence rate in people in the Great Plains region of the United States compared to the rest of the country and the reason is unknown.

Along with the introduction of WNV, the encroachment of Eastern Redcedar (ERC) has also posed as a problem in the Great Plains region of the United States. The encroachment has changed landscapes that once were natural grasslands into areas with completely enclosed canopy of ERC. This encroachment has decreased livestock grazing areas, changed water flow, and introduced new habitat for birds. Environmentally ERC takes its toll on the landscape by altering the way it is used. ERC is important to understand to gain the knowledge of how the landscape can play role in the nidus of infection, or how a virus is transmitted through the environment.

The results of this study has shed some light on how prominent landscape features in Oklahoma impacts the mosquito species which moves WNV in the environment. Understanding how the three parts of the disease transmission triangle (vectors, hosts, and pathogen) interact in a given environment, we can focus on ways to prevent or control future outbreaks of disease. This

study identified important aspects that relate to three questions: what mosquito communities are found in various ERC densities, how WNV *Culex* sp. vectors are interacting with ERC, and how the blood-fed mosquitoes are using ERC by comparing success of various trap styles. These three components helped to further identify the type of shelter that ERC provides for mosquitoes and the risk ERC may have in the community.

Our findings can provide helpful information for producers. Firstly, there are different types of mosquitoes in various densities of ERC. *Ae. albopictus* prefers higher density ERC and *Ps. columbiae* prefers grassland. *Cx. tarsalis* in Blaine county preferred ERC over grass sites. These three mosquito species are all vectors of multiple different pathogens and could be a danger to both people and livestock if not controlled. Secondly, we identified WNV infected *Culex* sp. in ERC. This further emphasizes the risk that if *Culex* sp. are using ERC and the trees are near people or livestock, in particular horses, then the risk of infection with WNV goes up. Finally, the stage that mosquitoes are using ERC is also important. The majority of mosquitoes were collected with CDC light traps, which uses CO_2 as an attractant. This means they are using ERC to navigate the terrain to seek out their hosts. Gravid traps were the second most successful, with female mosquitoes seeking water sources around ERC to lay their eggs. The last style, resting traps, had limited success but showed they use ERC to rest in after feeding.

This study can help with management efforts for producers. Finding what vector species are using ERC as well as the density they are inhabiting can control easier. Major vectors are using ERC, thus removal should be at the top of a producer's priority. If removal is too costly, pesticide applications can be made in those areas as well. Protecting the livestock from mosquitoes can be important not only from a disease perspective but also a nuisance aspect as well. If the animals external parasite burden is too high, a decline in production could occur, such as decreased weight gain, reduced milk production, or even a result of unhealthy offspring. Producers can use this information to better manage the pests around their livestock to help save them time and money.

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APPENDICES

Fig 1. CDC Light trap, without light, baited with two pounds of dry ice in ERC.

Fig 2. Gravid trap baited with fermented grass water in ERC.

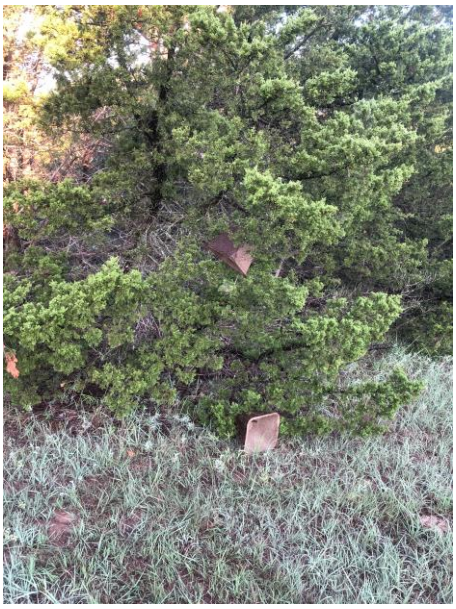

Fig 3. Resting fiber pot trap setup in ERC.

Fig 4. Resting bag and wire trap setup in ERC.

Fig 5. Resting trap setup with 32 gallon bucket in ERC.

Fig 6. Zone 3 site 6 ERC statistical analysis preparation.

VITA

Courtney Nicole Maichak

Candidate for the Degree of

Master of Science

Thesis: NATIVE INVASION OF EASTERN RED CEDAR (*JUNIPERUS VIRGINIANA*) IN US GREAT PLAINS AND THE THREAT OF WEST NILE VIRUS TRANSMISSION

Major Field: Entomology and Plant Pathology

Biographical:

Education:
Completed the requirements for the Master of Science in your major at Oklahoma State University, Stillwater, Oklahoma in July 2021.

Completed the requirements for the Bachelor of Science in Entomology at Oklahoma State University, Stillwater, Oklahoma in December 2017.

Experience:
Professional Memberships:
Entomological Society of America, Southwestern Branch

Publication:

Noden, BH, Loss, SR, **Maichak, C**, Williams, F. 2017. Risk of encountering ticks and tick-borne pathogens in a rapidly growing metropolitan area in the U.S. Great Plains. Ticks and Tick-borne Diseases 8: 119-124.

Presentations:

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