



Original Contribution

Invasive Plants as Foci of Mosquito-Borne Pathogens: Red Cedar in the Southern Great Plains of the USA

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Abstract: West Nile virus (WNV) is the most significant mosquito-borne disease affecting humans in the United States. Eastern redcedar (ERC) is a native encroaching plant in the southern Great Plains that greatly alters abiotic conditions and bird and mosquito populations. This study tested the hypotheses that mosquito communities and their likelihood of WNV infection differ between ERC and other habitats in the southern Great Plains of the United States. We found support for our first hypothesis, with significantly more *Culex tarsalis* and *Culex erraticus* in ERC than deciduous and grass habitats. Mosquito communities in Central Oklahoma were more diverse (21 species) than western Oklahoma (11 species) but this difference was not associated with vegetation. Our second hypothesis was also supported, with significantly more WNV-infected *Culex* from ERC in both regions, as was our third hypothesis, with significantly more *Culex tarsalis* and *Culex pipiens* collected in ERC than other habitats in urban areas. The connection of mosquito-borne disease with invasive plants suggests that land management initiatives can affect human health and should be considered in light of public health impact. Evidence from other vector-borne disease suggests invasive plants, both in the Great Plains and globally, may facilitate the transmission of vector-borne pathogens.

Keywords: Invasive plants, *Culex pipiens*, *Culex tarsalis*, *Juniperus virginiana*, Public health, West Nile virus

INTRODUCTION AND PURPOSE

Vector-borne disease transmission revolves around the concept of ‘nidality’ (Pavlovsky 1966). In any given arthropod vector–host–pathogen system, the risk of pathogen transmission will vary depending on the diversity, abundance, and distribution of host and vector species

within a given landscape (i.e., the ‘nidus of infection’ (Reisen 2010)). One critical aspect to predict spatial risk of transmission is to understand how specific vector species utilize specific habitats in a given region (Reisen 2010). This allows us to pinpoint nidi of infection within a given habitat and provides a means to prevent or control transmission with minimal damage to the environment.

Habitats occupied by blood-sucking arthropods are in a constant state of flux as anthropogenic environmental changes alter the ecology and transmission of vector-borne diseases (Foley et al. 2005; Allen et al. 2017). The expansion of native and non-native woody plant species in grasslands

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and shrublands (i.e., woody plant encroachment; WPE) is a major type of anthropogenic land-use change (Engle et al. 2008; Eldridge et al. 2011; Nackley et al. 2017). WPE is driven by changes in land management (e.g., lack of prescribed fire, grazing regimes, abandonment), landscape fragmentation, and climate change (Eldridge et al. 2011; Nackley et al. 2017; Venter et al. 2018). WPE changes abiotic conditions (e.g., temperature, moisture), biodiversity and species composition of arthropods and wildlife and large-scale vegetation cover (Ratajczak et al. 2012; Archer et al. 2017; Acharya et al. 2018).

One understudied consequence of WPE is its potential underlying role in influencing the nidi of transmission for vector-borne diseases. For example, in the southern Great Plains (Oklahoma, Kansas & Texas), the main encroaching plant is eastern redcedar (*Juniperus virginiana*; ERC) (Twidwell et al. 2018; Zou et al. 2018). Expanding at rates of 40 km² per year (Wang et al. 2018), eastern redcedar is increasing throughout the Great Plains region (Zou et al. 2018). Growing in height at a steady rate of 0.5 m/year (Engle and Kulbeth 1992), invading trees form dense woodland areas (Engle et al. 2008; Wang et al. 2018). As ERC encroachment substantially changes abiotic conditions (e.g., temperature, humidity, wind speeds) and water cycling (Caterina et al. 2014; Acharya et al. 2018), it affects the abundance, diversity, and species composition of regional plants, arthropods, and wildlife (mammals and birds) (Horncastle et al. 2005; Frost and Powell 2011; O'Brien and Reiskind 2013). Significantly influencing mosquito assemblages and providing nutritive inputs for larval mosquitoes (Reiskind and Zarrabi 2011; O'Brien and Reiskind 2013), ERC also affects the distribution of two important tick species (Noden and Dubie 2017) as well as horseflies (Sherrill 2019). While other invasive plants, mainly within tree habitat contexts, have been shown to influence vector and host distribution in the USA (Allan et al. 2010; Reiskind et al. 2010; Williams and Ward 2010; Gardner et al. 2017; Adalsteinsson et al. 2018), no mosquito-focused study has associated changes in vector distribution with levels of WPE by an invasive plant species throughout a region.

West Nile virus (WNV) is the most significant mosquito-borne disease affecting humans in the U.S.A (Curren et al. 2018; Rosenberg et al. 2018) with the Great Plains having the highest cumulative incidence of WNV human cases and neuroinvasive disease (Reimann et al. 2008; Petersen et al. 2013). However, it remains one of the least-studied regions with regard to the ecology of WNV vectors

and hosts and their associations with landscape factors (Bolling et al. 2009; Kent et al. 2009; Lindsey et al. 2008; Paull et al. 2017; Bradt et al. 2019). The confluence of components in Oklahoma provides an opportunity to focus on the nidality of WNV as it relates to ERC. First, WNV is endemic with occasional outbreaks (Johnson et al. 2015). Second, ERC is expanding in the state at rates 5–7 × greater than other areas of the United States (Zou et al. 2018; Wang et al. 2018). Third, *Culex tarsalis* is associated with ERC in central Oklahoma (O'Brien and Reiskind 2013). While these individual components exist, there is a significant gap in understanding how land cover (ERC, in particular) influences mosquito vectors of WNV in the southern Plains and, specifically, how this predominant form of WPE contributes to the occurrence of a nidus of WNV infection.

In this study, we tested three hypotheses involving relationships between adult mosquito species diversity and their habitat preferences across urban and rural habitats in central and western Oklahoma, using a series of field comparisons at a landscape scale. First, we tested whether ERC alters mosquito assemblages, favoring known vectors of WNV, across different landscapes in the southern Great Plains. Second, we tested whether ERC creates nidi of transmission, seen in higher likelihood of WNV infection in mosquitoes from ERC compared to other habitats. Third, we tested whether mosquito assemblages, again favoring WNV vectors, differ in the southern Great Plains with proximity/density of human habitation, making the connection between vector and host necessary for the natural nidality of WNV.

METHODS

Study Design

We conducted this study in two Eastern redcedar expansion areas: north central Oklahoma and western Oklahoma (Fig. 1). ERC expansion areas were defined using published data (Wang et al. 2018). We chose sites using Google Maps (Google, Mountain View, CA) then contacted site landowners who provided verbal consent for mosquito trap placement.

Mosquito Trapping

In each expansion area, eight sites were chosen as a replication unit: four urban and four rural (Fig. 1). Because of geographical distances and logistics, central Oklahoma was

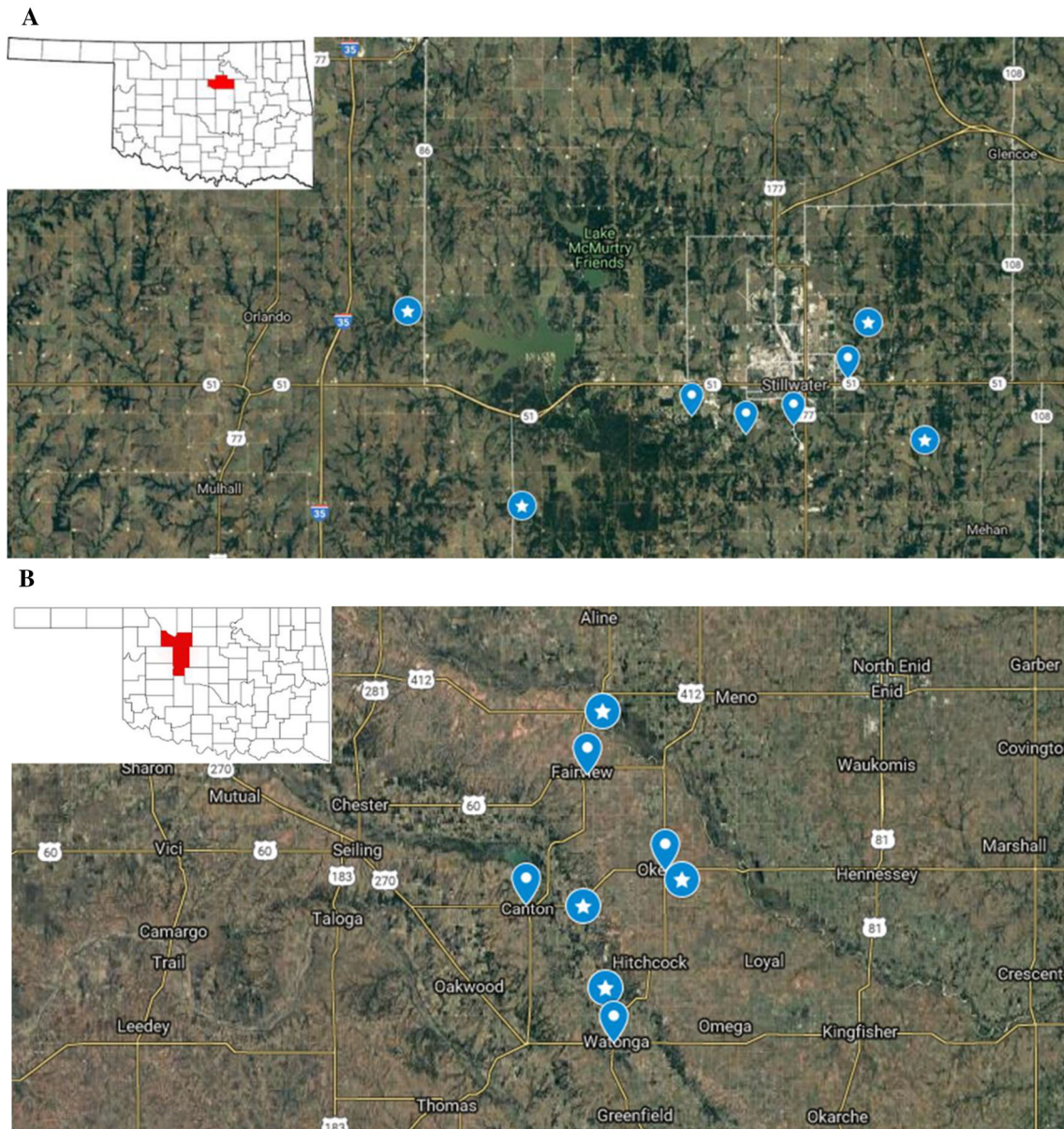


Figure 1. Trapping locations for mosquitoes in different canopy types in central (a) and western (b) Oklahoma. Stars = rural sites, Dots = urban sites.

surveyed in 2017 and western Oklahoma was surveyed in 2018. Each site contained four different habitat types: open canopy ERC (single tree or only touching another eastern redcedar on one side), closed canopy ERC (eastern redcedar trees touching at least two other eastern redcedar trees), deciduous trees (hard wood trees such as oak, ash, or elm), and open grass (an area of grass at least 50 m from any tree). In total, we had a total of 64 trapping locations (8 sites \times 4 traps per site \times 2 expansion areas/years). All traps were more than 100 m away from the other to maintain independence of trapping site. In anticipation of

expansion in Western Oklahoma, monthly trapping in 2017 occurred at a rural site in Major county involving 8 traps (2 traps per canopy type—separated $>$ 100 m).

We used CDC Mini Light Traps (Bioquip, Rancho Dominguez, CA) with lights removed together with CO₂ released from dry ice in modified insulated coolers were hung 1.5 m off the ground at all sites (O'Brien and Reiskind 2013). Traps were hung mid-afternoon and picked up between 8 and 9am the next morning. We collected adult female mosquitoes by trap type and location and stored them at -20°C until we identified them to species

using Darsie and Ward (2005) and stored separately in 7-dram vials at -20°C by species, collection date, trapping location, and canopy site. As all sites are located in a hybrid zone, all references to *Culex pipiens* L. denote the *Culex pipiens/quinqüefasciatus* complex as we were not able to distinguish these two species.

Pathogen Testing

West Nile virus testing was conducted by the Army Public Health Command-Atlantic (APHC) (Bethesda, Maryland) using *Culex pipiens* complex and *Culex tarsalis* mosquitoes separated into pools of 10 or less by location, collection date and canopy type and then shipped overnight. Pools of 2017 mosquitoes were sent on freezer packs, but not 2018 pools, because it does not affect virus detection (Turell et al. 2002). Samples were tested using qRT-PCR with 3'NC primers/TaqMan probe; any sample with a Ct value greater than 0 was tested in triplicate using qRT-PCR and primers and TaqMan probe. Samples positive with screening assay (Lanciotti et al. 2000) and confirmation assay (at least 2 out of 3 replicates) were given WNV Status of 'DETECTED'. *Culex pipiens* complex and *Culex tarsalis* were the only species tested because of cost considerations as well as being the main WNV vectors in Oklahoma (Noden et al. 2015).

Statistical Analysis

As this study was focused on testing a spatial hypothesis involving mosquito communities in specific habitats and not phenology, we combined total catches per mosquito species for each habitat in each location across ten (2017) and eight (2018) sampling periods. We used SAS JMP pro 15 (SAS) to analyze these data. Over the 10 sampling periods in 2017 (June 15 to October 26), there were no trap failures ($n = 320$ trap nights (40 nights \times 2 sites/night \times 4 traps/site)). Sampling ended early with only 8 sampling periods (no trap failures) in 2018 (June 23 to October 4) due to catastrophic regional flooding ($n = 256$ trap nights (16 nights \times 4 sites/night \times 4 traps/site)). Assessment of temporal differences in the two regions included one-way ANOVA by region.

One-way ANOVA was used to test for differences across canopy type using two measures of mosquito diversity to ensure a more comprehensive analysis: (1) The Shannon-Weiner index, a measure of species diversity in a

particular community that incorporates measures of abundance (numbers) and evenness (uniqueness); and (2) Shannon evenness, a measure of how similar species numbers is in a given habitat with 0 being complete dominance by one species and 1 being complete evenness of species (Martins et al. 2018; Spence Beaulieu et al. 2019).

To analyze mosquito abundance-related hypotheses, raw mosquito species count was divided by number of trap nights to control for differences in sampling efforts and a square root transformation was performed to normalize the data. To ensure adequate numbers throughout the trapping period, we only ran statistics on mean trap night abundances of each species with greater than 200 specimens. Statistical comparisons were conducted using two-way ANOVA by the four canopy types and urban/rural trap sites by region. Kruskal–Wallis rank sums test was used because of the non-normal nature of the data and Dunn's test to compare species abundance by habitat type. Sites were considered 'rural' if they were at least three miles outside of a small town ('urban cluster') while 'urban' sites were within the perimeter of the town. This analysis was followed up with generalized linear mixed models (GLMMs) (SPSS 26.0) to test the hypothesis that the abundance of each mosquito species differs across habitat type. Canopy type and urban/rural setting were input as fixed effects with the random effect by clusters of sites where collections occurred. Mosquito abundance of species greater than 200 collected was regressed against habitat type using negative binomial errors and log link functions with canopy as the fixed function using a Satterthwaite approximation for small datasets and a robust estimation to handle violations of model assumptions.

WNV mosquito infection rates were calculated on pooled mosquitoes as minimum infection rates (MIR) per 1000 females using PooledInfRate, a CDC-provided Microsoft Excel add-in (Biggerstaff 2006). Mosquito infection data were parsed by ERC expansion area/year. MIR was used instead of MLE (maximum likelihood estimate) because of small, uneven pool numbers (Fryxell et al. 2014). We performed two nonparametric tests to compare WNV MIR across habitat type in SAS JMP Pro (15.0). Kruskal–Wallis test was chosen for the non-normal nature of the data and Dunn's test was used to identify which habitat pairs differed for WNV MIR. We further evaluated differences between habitat types and presence of WNV-infected pools (by accounting for differences in mosquito pool si-

zes) using a likelihood test that compared the probability of WNV-infected mosquito in differing habitat types (Spence Beaulieu and Reiskind 2020).

RESULTS

Mosquito Diversity and Abundance

A total of 5, 273 adult female mosquitoes were collected from central (2,864) and western (2,409) Oklahoma (Table 1). Of the 23 species collected, eight comprised 96.3% of the total collected: *Culex tarsalis* (25.2%), *Culex erraticus* (16.8%), *Psorophora columbiae* (13.7%), *Aedes vexans* (12.0%), *Psorophora cyanescens* (12.0%), *Culex pipiens* complex (9.0%), *Anopheles quadrimaculatus* (3.9%), and *Aedes albopictus* (3.0%). The majority species varied by region/year of collection with 80% of those collected in the central region consisting of *Culex erraticus* (28.4%), *Aedes*

vexans (17.7%), *Culex tarsalis* (12.1%), *Culex pipiens* complex (11.3%), and *Psorophora columbiae* (9.4%) and 78% in the western region consisting of *Culex tarsalis* (40.8%), *Psorophora columbiae* (18.6%), and *Psorophora cyanescens* (18.6%). In the western expansion area where collections occurred in 2017, 302 mosquitoes (16 species) were collected of which *Cx. tarsalis* (63.9%) predominated followed by *An. quadrimaculatus* (9.9%), *Ps. columbiae* (6.9%) and *Ae. vexans* (4.6%).

The mosquito diversity in the central region (21 species) was higher than the western region (11 species). While no association existed between Shannon-Weiner diversity and habitat in the central region ($F = 1.04$, $df = 3$, $P < 0.387$), a significant association did exist in the western region ($F = 3.26$, $df = 3$, $P < 0.036$), with a higher diversity in grass than deciduous sites ($Z = 2.425$, $P < 0.0459$) (Fig. 2a). Additionally, there was a significant association between Shannon evenness and habitat in western Oklahoma

Table 1. Summary of Mosquitoes Collected in Rural and Urban Sites in Central and Western Oklahoma.

	Central			Western			
	Rural	Urban	Total	Rural	Urban	Total	Total
<i>Ae. albopictus</i>	57	16	73	48	35	83	156
<i>Ae. epactius</i>	4	0	4	0	0	0	4
<i>Ae. sollicitans</i>	10	11	21	0	0	0	21
<i>Ae. trivittatus</i>	51	26	77	4	1	5	82
<i>Ae. vexans</i>	165	342	507*	99	30	129	636
<i>An. barberi</i>	1	0	1	0	0	0	1
<i>An. pseudopunctipennis</i>	1	0	1	0	0	0	1
<i>An. punctipennis</i>	2	2	4	0	0	0	4
<i>An. quadrimaculatus</i>	48	97	145	42	18	60	205
<i>Cx. coronator</i>	0	0	0	0	0	4	4
<i>Cx. erraticus</i>	259	556	815*	37	37	74	889
<i>Cx. pipiens</i>	169	155	324	67	86	153	477
<i>Cx. salinarius</i>	0	4	4	0	0	0	4
<i>Cx. tarsalis</i>	121	227	348	513	469	982*	1330
<i>Cx. territans</i>	6	32	38	0	0	0	38
<i>Or. signifera</i>	0	1	1	0	0	0	1
<i>Ps. ciliata</i>	5	22	27	14	6	20	47
<i>Ps. columbiae</i>	96	175	271	269	182	451*	722
<i>Ps. cyanescens</i>	132	55	187	360	88	448*	635
<i>Ps. ferox</i>	6	4	10	0	0	0	10
<i>Ps. mathesoni</i>	4	5	9	0	0	0	9
<i>Ur. sapphirina</i>	1	0	1	0	0	0	1
TOTAL			2868			2409	5277

*Mean trap night abundance significantly differs between region $P < 0.05$.

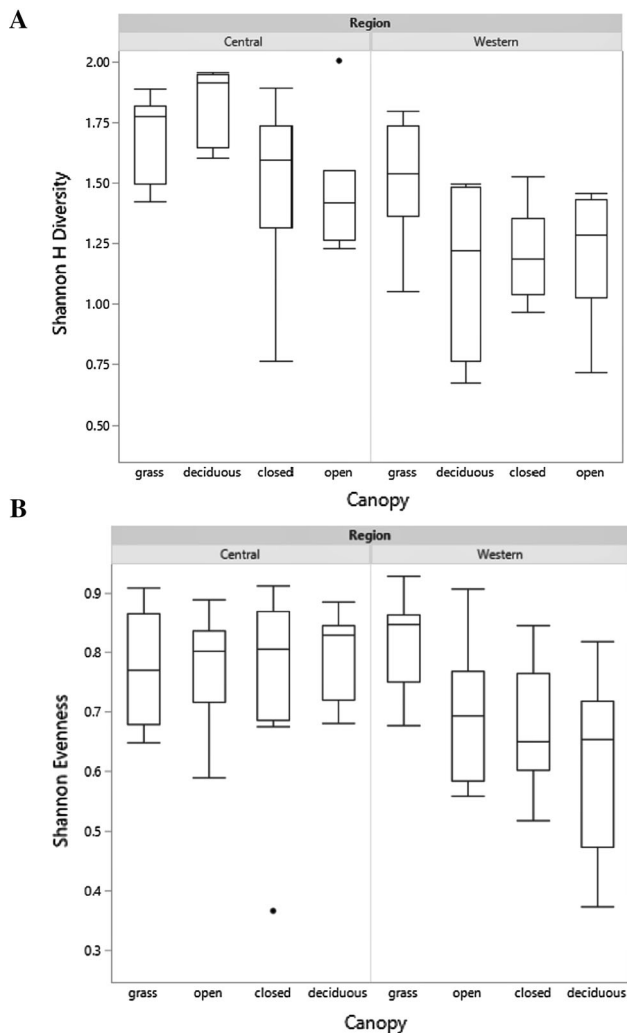


Figure 2. Boxplot diversity comparisons between habitat-types. **a** Shannon-Weiner diversity and **b** Shannon evenness by habitat type. In each boxplot, the mean is represented as a black line with the upper and lower quartiles making the box and whiskers being within $1.5 \times$ the interquartile range. Outliers are plotted as dots

($F = 3.73$, $df = 3$, $P < 0.023$) (Fig. 2b) with a significantly higher mean evenness values for grass than deciduous habitats ($Z = 2.746$, $P < 0.0362$). Mosquito diversity did not differ between rural and urban sites in central (Shannon-Weiner: $F = 0.785$, $df = 1$, $P < 0.3828$; Evenness: $F = 3.84$, $df = 1$, $P < 0.0595$) or western (Shannon-Weiner: $F = 0.0001$, $df = 1$, $P < 0.9910$; Evenness: $F = 2.83$, $df = 1$, $P < 0.1028$) Oklahoma. There were also no relationships between diversity indices and canopy types between urban and rural areas in the two regions (data not shown).

Temporal variation in time but not region was apparent between *Culex tarsalis* populations in different habitats (Fig. 3). *Culex tarsalis* populations in all canopy

types peaked between September 7 and 18 in central Oklahoma ($F = 9.54$, $P < 0.0001$), while peaking between August 18 and September 8 ($F = 6.64$, $P < 0.0001$) in western Oklahoma except in grass which peaked in July. *Culex tarsalis* were still collected in all habitats, considerably less in grass, in October in western Oklahoma.

Species-Habitat Spatial Relationships

Mean trap night abundance of *Culex tarsalis* significantly associated with habitat type ($F = 5.54$, $df = 3$, $P < 0.0020$) in the study as a whole but associations differed by region. In central Oklahoma ($X^2 = 8.37$, $df = 3$, $P < 0.039$), *Culex tarsalis* was more likely associated with closed ERC than deciduous ($Z = 2.26$, $P < 0.0235$) and grass ($Z = 2.42$, $P < 0.0156$) habitat. In western Oklahoma ($F = 8.89$, $df = 3$, $P < 0.0003$), *Culex tarsalis* was significantly more likely in closed ERC ($Z = 3.45$, $P < 0.0017$), open ERC ($Z = 3.01$, $P < 0.0077$) and deciduous ($Z = 2.47$, $P < 0.0409$) habitats compared with grass. Of the other species collected, *Psorophora columbiae* was associated with grass habitats in the central region ($F = 4.21$, $df = 3$, $P < 0.014$) with significant differences between grass and closed ERC habitat ($Z = 3.09$, $P < 0.012$). *Culex erraticus* was associated with closed ERC habitat in the central region ($X^2 = 8.17$, $df = 3$, $P < 0.0425$) with significant differences with grass habitat ($Z = 2.65$, $P < 0.048$). The GLMMs of species segregation in relation to habitat type supported the data from the trap night abundance analyses. For both regions, *Culex tarsalis* more likely in ERC habitats compared with grass and deciduous ($F = 8.342$, $df = 3$, $P < 0.0000$), while *Cx. erraticus* was more likely in closed ERC than grass ($F = 4.71$, $df = 3$, $P < 0.003$) (Table 2).

Overall mean trap night abundance for *Cx. tarsalis* was also significantly associated with urban habitats ($F = 3.74$, $df = 3$, $P < 0.0223$) with significant association with closed ERC ($Z = 2.89$, $P < 0.0038$) compared with grass habitat (Supplemental Fig. 1). The significant relationships occurred in western Oklahoma ($X^2 = 8.73$, $df = 3$; $P < 0.0389$) where *Cx. tarsalis* in urban areas was more associated with closed ERC ($Z = 2.17$, $P < 0.0294$) and deciduous ($Z = 2.17$, $P < 0.0294$) habitat than grass. There was also a positive trend with open ERC compared with grass but it was not significant ($Z = 1.89$, $P < 0.0591$). Overall mean trap night abundance for *Cx. pipiens* was also significantly associated with urban habitats ($F = 4.16$, $df = 3$, $P < 0.0147$) with significant association with closed ERC compared with deciduous ($Z = 2.66$,

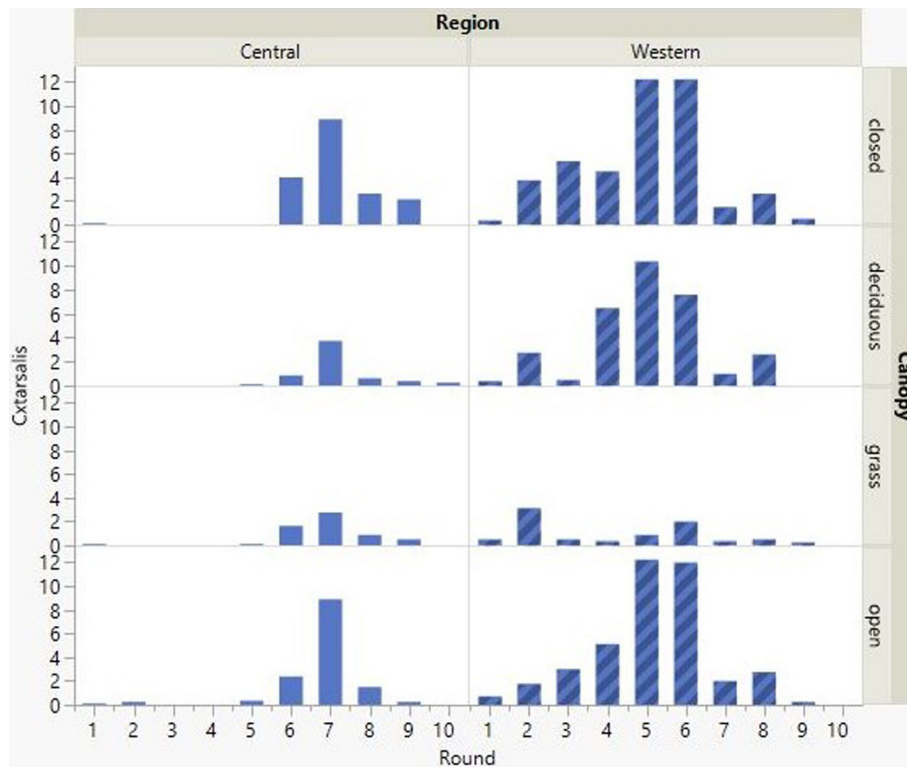


Figure 3. Mean *Culex tarsalis* abundance by canopy in central and western Oklahoma by collection round.

Table 2. GLMM Results for *Culex tarsalis* and *Culex erraticus* by Canopy Habitat and Location of Collection.

Model	Fixed effects	Coefficient	Std. Error	t	Sig	95% CI for coefficient	
						Lower	Upper
<i>Culex tarsalis</i>	Intercept	1.061	0.3181	3.336	0.004	0.395	1.728
	Canopy						
	Open ERC	- 0.222	0.2817	- 0.788	0.431	- 0.775	0.331
	Grass	- 1.376	0.2938	- 4.683	0.000	- 1.953	- 0.799
	Deciduous	- 0.639	0.2847	- 2.244	0.025	- 1.198	- 0.08
	Closed ERC ^a						
<i>Culex erraticus</i>	Location						
	Urban	0.134	0.2044	0.653	0.514	- 0.268	0.535
	Rural ^a						
	Neg. Binomial	5.279					
	Intercept	1.399	0.3229	4.333	0.001	0.715	2.083
	Canopy						
Open ERC	- 0.469	0.4029	- 1.165	0.245	- 1.262	0.323	
Grass	- 1.536	0.4145	- 3.705	0.000	- 2.351	- 0.72	
Deciduous	- 0.696	0.4045	- 1.721	0.086	- 1.492	0.1	
Closed ERC ^a							
Neg. Binomial	6.167						

^aDenotes the reference category.

$P < 0.0473$) habitat. The significant relationship occurred in central Oklahoma ($F = 12.16$, $df = 3$, $P < 0.0006$) where *Cx. pipiens* in urban areas was more associated with closed ERC compared with deciduous ($Z = 3.02$, $P < 0.0151$) habitat. Of the other species collected, *Ae. vexans* ($F = 12.41$, $df = 3$, $P < 0.0014$) and *An. quadrimaculatus* ($F = 5.27$, $df = 3$, $P < 0.0289$) were associated with rural habitat in western Oklahoma but there was no association with any particular habitat. *Aedes albopictus* was not associated with any particular habitat in central ($F = 1.66$, $df = 1$, $P < 0.2068$) or western ($F = 0.245$, $df = 1$, $P < 0.6242$).

Association of Habitat with Mosquito Infection

Eight of nine WNV-positive mosquito pools were collected in closed or open ERC habitat (Supp Fig. 2). The other WNV-positive pool was collected in a deciduous habitat in western Oklahoma. There was a higher probability of collecting WNV-infected *Culex* mosquitoes in ERC habitats (LRT test: $X^2 = 8.510$, $P < 0.0366$) but location of collection (urban or rural) was not significant (LRT test: $X^2 = 0.130$, $P < 0.719$). The highest MIR (10.8/1000 mosquitoes) in western Oklahoma was in open ERC with the second highest (7.3/1000 mosquitoes) in closed ERC due to the apparent proclivity of *Cx. tarsalis* for encroached areas (Table 3). All WNV-positive mosquito pools were collected between August 25 and September 25 in all four

geographical areas sampled, matching the peak periods of abundance for *Cx. tarsalis*.

DISCUSSION

We found that invasive ERC altered mosquito assemblages, particularly *Cx. tarsalis*, across the southern Great Plains region. In central Oklahoma, more *Cx. tarsalis* per trap night were collected in closed ERC compared with grass or deciduous habitat, while in western OK, *Cx. tarsalis* was more associated with all habitats compared with grass. This preference increased the likelihood of encountering WNV-infected *Culex* mosquitoes in ERC habitats. This preference was not dependent on whether the site was urban or rural or open or closed ERC. Finally, we found that *Cx. tarsalis* and *Cx. pipiens* were both associated with ERC habitats in urban areas across the region. This is the first evidence that links WPE with the most important mosquito-borne disease in the continental United States in the region most affected by neuroinvasive WNV (Rosenberg et al. 2018). These results have important ramifications for human risk of encountering a WNV-infected mosquito in the southern Great Plains, and being infected with WNV.

Mosquito species assemblages in the southern Great Plains clearly differed in diversity in the humid, wetter central cross-timbers habitats (21 species) compared with arid, drier western regions (11 species) (OCS 2016). Of importance to arbovirus transmission, more *Cx. pipiens* and *Cx. erraticus* were collected in central Oklahoma, while

Table 3. West Nile Virus Minimal Infection Rates (MIR) in Pooled *Culex tarsalis* and *Culex pipiens* Complex Collected in Different Canopy Types in Central and Western Oklahoma.

Canopy type	MIR	Lower Limit	Upper Limit	# pools tested	# positive pools	Total # mosq tested
<i>Culex tarsalis</i>						
Western Oklahoma						
Closed ERC	7.35	0	13.89	76	3	343
Open ERC	10.78	0.33	24.83	65	4	371
Deciduous	3.70	0	11.64	53	1	270
Grass	0	N/A	N/A	34	0	126
<i>Culex pipiens</i> complex						
Central Oklahoma						
Closed ERC	6.85	0	20.23	35	1	146
Open ERC	0	N/A	N/A	31	0	109
Deciduous	0	N/A	N/A	23	0	54
Grass	0	N/A	N/A	19	0	28

Minimal Infection Rate (MIR) measures the level of mosquito infection (number of WNV-positive pools/ all mosquitoes tested \times 1000).

significantly more *Cx. tarsalis* were collected with western Oklahoma. Mosquito distribution is highly resource driven, mainly impacted by availability of hosts for bloodmeals, flowering plants for nectar, and appropriate habitats for larval development (Reiskind et al. 2017). If there is a particular habitat preference by a specific species of mosquito, this is most likely due to many of necessary resources available around that habitat type. In the case of the preference of *Culex* mosquitoes for ERC, the habitats which contribute to a highly diverse mosquito community in central Oklahoma are contrasted with the open-spaced, arid-pastured, vegetation-limited habitat in western Oklahoma where larval breeding sites may be kilometers from a blood meal host or plant nectar site. The reduction in vector diversity may also contribute to an increase in WNV risk as 'hot-spots' may occur in specific sites dominated by one or two species (Lyimo and Ferguson 2009). The availability of an evergreen tree which transpires 24 L of water daily (Caterina et al. 2014) almost year-round, may provide a humid oasis in which mosquitoes can rest and sequester from wind (significant decrease of wind compared with other habitats (Cote, unpublished data)). Within this oasis, mosquitoes can obtain bloodmeals from birds which use the ERC for food, shelter, nesting, and roosting (Coppedge et al. 2001; Kilpatrick et al. 2006a, b; Thiemann et al. 2011) and nectar from surrounding wild flowers. Bird species known to prefer ERC in different parts of the year are known reservoir hosts for WNV, the northern cardinal (*Cardinalis cardinalis*) and the common robin (*Turdus migratorius*), (Coppedge et al. 2001; Godsey et al. 2005; Kilpatrick et al. 2006a; Dusek et al. 2009; Hamer et al. 2009). In the case of the current study, a lower diversity of mosquito community in ERC was linked with a higher likelihood of encountering WNV-infected *Culex* mosquitoes. The lower diversity of mosquito species in the ERC system may be enhancing the opportunities of *Culex* mosquitoes to find the resources necessary to thrive in an otherwise inhospitable environment. This is not always the case, however, as others have demonstrated that decreases in mosquito diversity can be directly associated with decreased pathogen prevalence in local mosquitoes and hosts (Spence Beaulieu and Reiskind 2020). Not enough WNV-infected mosquitoes were collected in the present study to identify relationships between diversity indices.

The importance of how ERC impacts the mosquito community is further demonstrated by the increased trap night abundance of *Culex* mosquitoes in urban areas compared with rural areas in central and western regions.

More specifically, the significantly higher abundance of *Cx. pipiens* and *Cx. tarsalis* in urban areas of central and western Oklahoma, respectively, occurred in closed ERC. The lack of relationship between species diversity in urban and rural sites indicated that the effects of factors associated in larger cities (Reiskind et al. 2017; Spence Beaulieu et al. 2019) are limited in small urban areas. However, more *Culex* species involved with WNV transmission in Oklahoma (Noden et al. 2015) preferred to use closed ERC habitat in urban areas. This is important from the perspective of developing integrated mosquito management in the region and protecting people from infection. Any mosquito management program developed in regions of the Great Plains where ERC is expanding (Zou et al. 2018) must take into account the preference of this tree by medically-important mosquito vectors. *Aedes albopictus*, however, did not exhibit a preference for habitat or urban landscape in any region. This major vector of human and animal pathogens in Oklahoma does not appear to be influenced by the habitat types measured and needs a separate focus in any integrated mosquito management program.

The current study has demonstrated that WNV-infected mosquito species in the southern Great Plains preferentially associate with two types of ERC and closed ERC, in particular. This finding has public health implications for reducing risk of exposure, particularly in the small urban areas found throughout the region. The economic and public health concerns regarding ERC have been known for decades as studies have detailed the impact of this invasive species on area hydrology, pasture health, livestock production, economic, plant, insect, bird, and mammal diversity, and cumulative human allergy burden (Zou et al. 2018; Flonard et al. 2018). While land owners have been increasingly willing to eliminate ERC and participate in prescribed burning for control (Joshi et al. 2017; Symstad and Leis 2017), ERC is continuing to expand throughout the Great Plains at a rate of 40 km²/y (Wang et al. 2018) and is now expanding into areas of North and South Dakota (Zou et al. 2018). Together with the current study, we now know that at least three arthropod-borne pathogen systems utilize ERC as a nidus of infection: horseflies that transmit bovine anaplasmosis (Sherrill 2019) and tick vectors of bacterial pathogens, both established (*Derma-centor variabilis*) and expanding populations (*Amblyomma americanum*) (Noden and Dubie 2017; Noden et al. 2021). There is a need for targeted extension and outreach activities to educate specific segments of the general populations

as well as livestock producers regarding the benefits of ERC removal in rural and urban areas in the Great Plains. The possibility of reducing human cases of neuroinvasive WNV would produce public health cost savings in the region that would be well worth consideration.

CONCLUSION

Given the importance of ERC in the nidus of infection for WNV in the southern Great Plains, future studies are needed in several areas. Firstly, the nidus of infection needs to be parsed in detail to identify how ERC is involved in the maintenance of *Culex* mosquitoes. This will entail monitoring how varying concentrations of expanding ERC impact vector mosquito and host abundance as well as host feeding-choices together with host usage. Secondly, more studies are needed to address how ERC impacts the nidality of other disease systems (tick, horse fly) at the local level. Finally, future studies need to expand into other regions of the world where WPE is occurring to assess the impact (positive/negative) on the risk for particular vector-borne diseases.

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DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reason request.

DECLARATIONS

CONFLICT OF INTEREST The authors declare that they have no conflict of interest.

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