Effects of woody plant encroachment by eastern redcedar on mosquito communities in Oklahoma

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ABSTRACT: Woody plant encroachment into grasslands is occurring worldwide, affecting ecosystems in ways that likely influence mosquito-borne disease transmission. In the U.S. Great Plains, encroachment by eastern redcedar (*Juniperus virginiana*) (ERC) may be expanding conducive habitat for mosquitoes and their hosts, but few studies have evaluated associations between ERC encroachment and West Nile virus (WNV). To test the hypotheses that mosquito abundance and WNV-infected mosquitoes increase with increasing ERC cover, we collected mosquitoes in 32 sites in Oklahoma reflecting various ERC encroachment stages. We found support for our first hypothesis, as mean abundance of *Aedes albopictus* increased significantly with ERC cover. However, *Psorophora columbiae* and *Anopheles quadrimaculatus* abundance decreased with increasing ERC. There was no significant association with ERC for other mosquito species. We could not test our second hypothesis due to low WNV prevalence, but the only detected WNV-infected pool of mosquitoes (*Cx. tarsalis*) was collected in ERC. Our results suggest ERC encroachment increases abundance of at least one medically important mosquito species, but further research is needed to clarify how encroachment affects ecology of the entire WNV disease system through changes to vector and host communities, vector-host interactions, and thus disease transmission and prevalence. Understanding relationships between woody plant encroachment and the nidus of infection for mosquito-borne diseases will be crucial for targeting public health efforts, including land management activities that limit and/or eradicate woody plant encroachment, particularly in areas with high levels of disease risk. ***Journal of Vector Ecology* 47 (2): 179-187. 2022.**

Keyword Index: Woody plant encroachment, *Culex tarsalis*, *Aedes albopictus*, West Nile virus.

INTRODUCTION

West Nile virus (WNV) is the most significant mosquito-borne disease affecting humans in the U.S., causing regional epidemics and neuroinvasive disease in 1-2% of those infected (Curren et al. 2018, Rosenberg et al. 2018). Other mosquito-borne arboviruses pose regional threats, including those that infect animals and humans, including equine encephalitis viruses (Reeves et al. 1947, Randolph et al. 1994, Lindsey et al. 2018b), Jamestown Canyon virus (Pastula et al. 2015), and dengue, Chikungunya, and Zika viruses (Rosenberg et al. 2018). The Great Plains region of the U.S. has the highest cumulative incidence of WNV human cases and neuroinvasive disease (Curren et al. 2018), and new human arboviruses continually threaten this region through the southern U.S. border (Brunkard et al. 2007, Lindsey et al. 2018a, Adams et al. 2019). Throughout the Great Plains, mosquito species that are competent vectors for each of these arboviruses are present. Yet, the region is one of the least studied regarding the ecology of arbovirus vectors and hosts and their associations with human-caused environmental changes including land cover change, which greatly affects transmission of vector-borne diseases (Patz et al. 2008, Lindahl and Grace 2015), including mosquito-borne diseases like WNV (Kilpatrick 2011).

A major form of land cover change that is occurring in the U.S. and worldwide, and that has substantial implications for the nidus of transmission for vector-borne diseases (Reisen 2010), is woody plant encroachment (WPE) (Loss et al. 2022). WPE is an increase in woody plant cover in grasslands and savannas that occurs primarily due to fire suppression and that greatly changes abiotic conditions and populations and communities of plants, invertebrates, and vertebrate wildlife (Archer et al. 2017). Grasslands of the Great Plains are experiencing significant encroachment by many woody species, especially eastern redcedar (*Juniperus virginiana*; ERC), which is expanding faster in the southern Great Plains (Oklahoma, Texas, and Kansas) than anywhere in the U.S. (Zou et al. 2018). ERC is native to eastern parts of this region, but frequent fires historically confined it to rocky ravines and outcroppings. Starting in the 1930s, ERC's distribution expanded in the southern Great Plains, as the tree was widely planted in shelterbelts and for erosion control. Decades of fire suppression led to ERC widely expanding into grasslands, savannahs, and woodlands (Twidwell et al. 2013). Once established, ERC can convert grasslands into dense forests within 40 years (Briggs et al. 2002, Wang et al. 2018).

As with other forms of WPE, encroachment by ERC changes abiotic conditions (e.g., temperature, moisture, and wind) and animal populations (Coppedge et al. 2001b, Wang et al. 2021). Recent studies also indicate that this invasive tree is likely affecting populations of vectors including ticks,

mosquitoes, and horseflies (Noden and Dubie 2017, Noden et al. 2021a, 2021b). Most relevant to WNV, the two primary mosquito species involved with transmission of this pathogen in urban areas of the Great Plains, *Culex pipiens* and *Culex tarsalis*, have been documented to prefer resting in ERC (O'Brien and Reiskind 2013, Noden et al. 2015, 2021a). Furthermore, in more rural areas of the region, *Cx. tarsalis* and *Cx. erraticus* are more abundant, and WNV-infection of *Culex* species mosquitoes appears to be more prevalent in ERC-encroached areas compared to open grasslands and deciduous woodlands (Noden et al. 2021a). To further refine understanding of the effects of ERC encroachment on WNV, we evaluated how mosquito populations are impacted by varying levels of ERC encroachment across a large area in central and western Oklahoma. Specifically, we tested the following hypotheses: 1) abundance of mosquitoes increases with expanding ERC cover, and 2) WNV-infected mosquitoes are more likely to be collected in ERC than in grasslands.

MATERIALS AND METHODS

Study locations

Between June and September, 2020, we worked with landowners and Oklahoma State University (OSU) county extension personnel to establish 32 mosquito sampling sites across seven counties in central and western Oklahoma, where ERC is rapidly expanding (Figure 1) (Wang et al. 2017, 2018). To increase logistical efficiency, sampling sites were clustered in four zones including: nine sites near Stillwater, OK, with six at the OSU Range Research Station (Payne County) and three at a private pasture (Logan County) [Zone 1]; nine in west-central Oklahoma approximately 90 km northwest of Oklahoma City, with six in a private pasture near Watonga (Blaine County) and three in a private pasture near Okeene (Kingfisher County) [Zone 2]; six approximately 80 km southwest of Oklahoma City in a private pasture near

Binger (Caddo County) [Zone 3]; and eight in south-central Oklahoma, with four in a pasture near Joy (Murray County) and four in a pasture near Lindsay (McClain County) [Zone 4]. These 32 sites were selected by first using aerial imagery in Google Earth to identify candidate sampling areas in each zone. After landowners had expressed interest in providing land access for our project, we made ground-truthing visits during which we visually identified sites capturing varying levels of ERC encroachment and after field visits, we verified encroachment stages by calculating percent horizontal cover of ERC using satellite imagery in Google Earth. The selected sites ranged in size from approximately 0.3-0.5 ha; the open grassland sites and remaining areas of grassland within ERC-encroached sites varied somewhat regarding the plant community, but most were dominated by species typical to tallgrass and mixed grass prairies of the region (e.g., big bluestem [*Andropogon gerardii*], little bluestem [*Schizachyrium scoparium*], and Indiangrass [*Sorghastrum nutans*]).

Trapping protocol

Mosquitoes were collected every other week at each site between 8 June and 17 August 2020, with each site visit entailing one night of sampling. When the academic semester began at OSU, logistic limitations required us to adjust sampling intensity to once every three weeks at each site (between 31 August and 26 September 2020). For each site visit, two CDC light traps per site (Bioquip, Rancho Dominguez, CA) were hung 1.5 m above the ground; each trap was baited with two pounds of dry ice (CO_2) and lights were removed to reduce collection of non-target insects. Traps set in sites with ERC were tied directly to the outer branches of trees while traps set in grassland control sites were attached to a T-pole that was pushed into the ground. We used light traps because they are known to attract a high diversity of female host-seeking mosquitoes (Reiskind et al. 2017). Traps were set up by 15:00
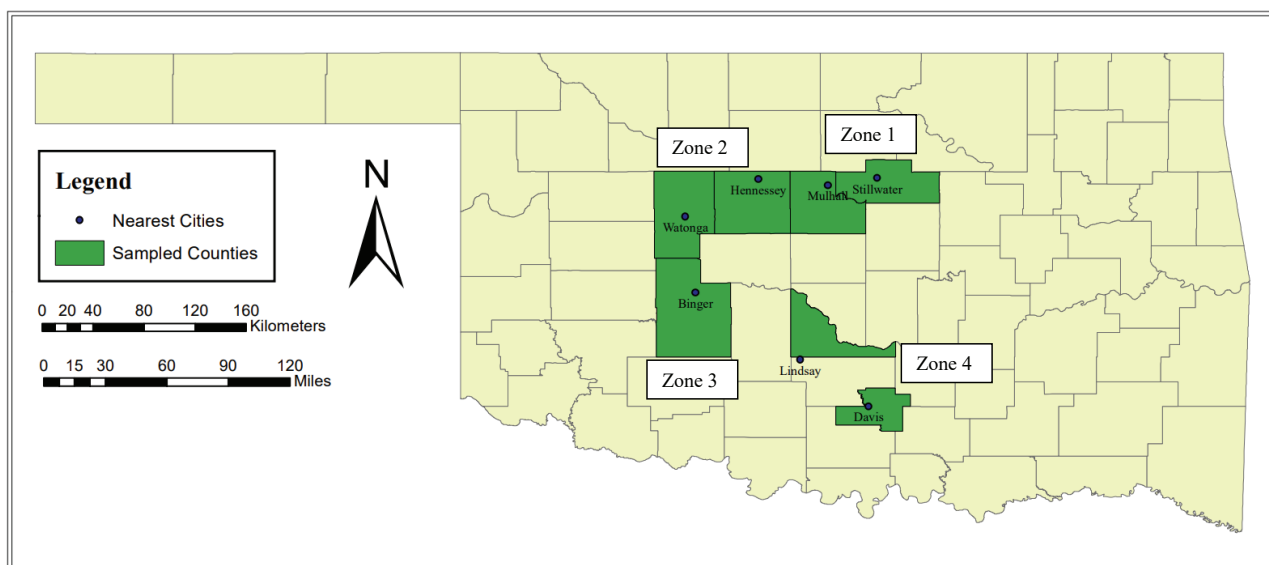

Figure 1. Map of Oklahoma showing the counties, four broad geographic zones (described in the main text), and specific locations (dots indicating nearest city/town) containing 32 study sites that captured different stages of eastern redcedar encroachment and at which mosquitoes were sampled between June and September, 2020 (ESRI ArcMap 10.8.1).

h and picked up the following day by 12:00 noon, ensuring collection of diurnal, crepuscular, and nocturnal species. In each site, traps were spaced ≥200 m apart to capture variability across sites and reduce the probability of trap sampling areas overlapping.

Species identification

At the time of collection, mosquitoes from the two traps at each site were pooled, and samples were labelled with the site name and date of site visit, immediately placed in a Whynter Portable Freezer (Whynter, Brea, CA) for quick euthanasia and storage during transportation, and later transferred to a -20º C freezer in the laboratory. Individual mosquitoes were identified using Darsie and Ward (2005). Upon identification, specimens were placed into snap cap vials (7-dram, Fisher Scientific, Hampton, NH) by species and returned to the -20° C freezer. Because southern Oklahoma is within a hybrid zone for *Culex pipiens* and *Culex quinquefasciatus,* we did not attempt to identify these taxa to the species level, instead identifying them as *Culex pipiens* L. complex (Harbach 2012).

West Nile virus testing

During sorting and identification, *Culex pipiens* and *Culex tarsalis* mosquitoes were pooled into groups of up to 25 individuals by site visit, site, and zone. RNA was extracted from mosquitoes using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) using the manufacturer's protocol. Real-time RT-PCR was performed on RNA extracted from mosquitoes using the QuantiTect Probe RT-PCR Kit (Qiagen), 25 pmol of WNV primers, 3.25 pmol of the probe, and 10 µl of the RNA extracted from the mosquitoes for a total reaction volume of 25 µl (Lanciotti et al. 2000, Hamer et al. 2011). Real-time PCR amplification of the reaction mix was performed on Rotor-Gene 6000 (Qiagen) consisting of a single cycle of 50º C for 30 min (reverse transcription) and 95º C for 15 min (hot start), followed by 40 cycles of 94º C for 30 s, 55º C for 1 min, and 68º C for 1 min. Result reports were created after cycles were completed. Positive controls were graciously provided by Dr. Gabriel Hamer (Texas A&M University).

Estimation of percent cover of eastern redcedar

To estimate site-level, percent horizontal cover of ERC, areas of ERC cover were visually identified for each site using leaf-off aerial images in Google Earth Pro. We then moved images to Blender Image Editor v2.8 (Hess 2010) and labelled cedar pixels as white. Python v3.4 (Python Software Foundation) was then used to modify images to black and white, and Blender Image Editor was used to calculate percent cover of ERC as the number of ERC pixels divided by total pixels in each site.

Data analysis

To test the hypothesis that increasing levels of ERC encroachment increase mosquito abundance, we analyzed the association between site-level percent cover of ERC and abundance of several medically important mosquito vector species that we found in our sites (*Ae. albopictus*,
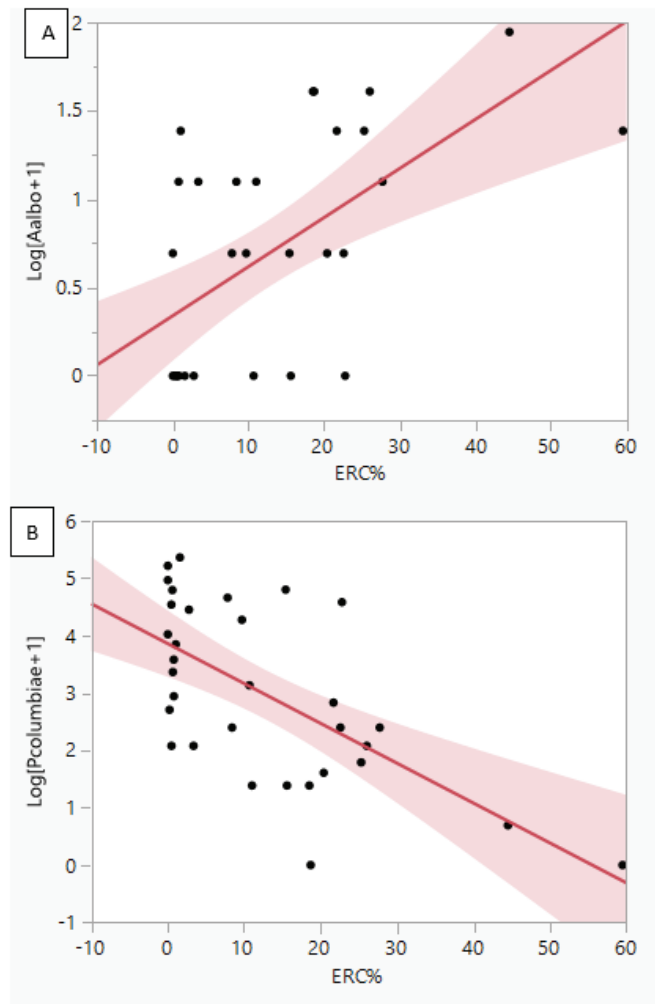

Figure 2. Results of linear regression analyses evaluating the relationship between percent cover of eastern redcedar and mean abundance (averaged across all visits for each site) of A) *Aedes albopictus* and B) *Psorophora columbiae,* collected between June and September, 2020 across 32 sites in Oklahoma.

An. quadrimaculatus, Cx. erraticus, Cx. pipiens, Cx. tarsalis, and *Ps. columbiae*). Statistical analysis was completed using SAS JMP Pro 15.2 (SAS Institute, Cary, NC, U.S.A.). First, to evaluate overall effects of ERC across the entire sampling season, we used linear regression analyses for which replicates were the 32 sites, response variables were mean abundances of each mosquito species averaged across all site visits (abundances were log+1 transformed to satisfy assumptions about normality and homogeneity of variance), and the fixed effect was percent ERC cover. Second, to also consider seasonal variation in mosquito abundance, we used linear regressions for which replicates were individual site visits (n=192 total site visits), response variables were log+1-transformed abundances of each mosquito species for each site visit, and fixed effects were percent ERC cover and site visit. For each analysis and response variable, the distribution of data, as visualized using quantile-residual plots and

Table 1. Total numbers of each mosquito species collected between June and September, 2020 across 32 study sites (in four different zones described in the main text) representing different stages of eastern redcedar encroachment in Oklahoma.

	Zone 1		Zone 2		Zone 3		Zone 4	Total
	Payne	Logan	Watonga	Okeene	Binger	Joy	Lindsay	
Aedes albopictus	15	3	7	0	17	1	3	46
Ae. canadensis	0	0	0	4	0	0	2	6
Ae. epactius	0	0	0	0	0	2	0	2
Ae. sollicitans	15	45	32	7	1	24	25	149
Ae. vexans	4	2	7	18	11	44	36	122
Ae. zoosophus	7	0	8	1	25	4	13	58
Anopheles crucians	0	0	0	0	0	2	0	2
An. punctipennis	3	7	176	0	5	2	9	202
An. quadrimaculatus	1	37	35	9	2	19	11	114
Culex coronator	1	1	11	1	19	4	0	37
Cx. erraticus	22	574	401	44	235	760	541	2,577
Cx. nigripalpus	2	4	18	0	3	74	6	107
Cx. perturbans	0	0	11	0	0	0	0	11
Cx. pipiens	2	5	60	6	5	22	6	106
Cx. salinarius	0	0	0	0	0	0	3	3
Cx. stigmatosoma	0	0	1	0	0	0	0	1
Cx. tarsalis	0	22	74	86	83	40	64	369
Psorophora ciliata	3	19	1	2	0	3	4	32
Ps. columbiae	144	196	590	395	36	99	76	1,536
Ps. cyanescens	18	33	43	65	2	14	61	236
Total	237	948	1,475	638	444	1,114	860	5,716

prediction profiler plots, suggested that transformed response variables met the assumption regarding normality. Formal analyses to test our second hypothesis (regarding the effect of ERC on numbers of WNV-infected mosquitoes) could not be completed due to low WNV detection in the samples.

RESULTS

Collection results

A total of 5,716 female mosquitoes were collected over 380 trap nights (Table 1) across all 32 sites, including six sites with 0% ERC cover, 13 with 0.1-20% cover, 11 with 21-40% cover, and two with 41-60% cover (specific ranges of ERC cover for each geographic zone studied were: 0-59.5% for Zone 1, 0-22.8% for Zone 2, 0-26% for Zone 3, and 0-22.6% for Zone 4). Over the course of the study, there were four trap failures involving rodents (n=2), cows (n=1), and a storm (n=1). The most mosquitoes were collected in zone 2 (n=2,113 (37.0%)) followed by zone 4 (n=1,974 (34.5%), zone 1 (n=1,185 (20.7%), and zone 3 (n=444 (7.8%) (Table 1, Figure 1). Of the medically important mosquitoes, the most common species was *Cx. erraticus* (n=2,577), followed by *Psorophora columbiae* (n=1,536), *Cx. tarsalis* (n=369), *An. quadrimaculatus* (n=114), *Cx. pipiens* (n=106), and *Aedes albopictus* (n=46) (Table 1).

Relationships between mosquito abundance and percent ERC cover

Our first set of analyses, which treated sites as replicates and included average mosquito abundances across all site visits as response variables, indicated that abundance of *Ae. albopictus* increased with increasing ERC cover (R^2=0.36, F ratio=17.34, df=31, P=0.0002) (Figure 2A) and that *Ps. columbiae* abundance decreased with increasing ERC cover (R^2=0.41, F ratio=13.61, df=31, P<0.0001) (Figure 2B). This analysis found no significant relationship between ERC cover and abundance for all other species evaluated, including *An. quadrimaculatus* (R^2=0. 10, F ratio=3.38, df=31, P=0.0761), *Cx. erraticus* (R^2=0.08, F ratio=2.59, df=31, P=0.1176), *Cx. pipiens* (R^2=0.03, F ratio=0.89, df=31, P=0.3517), and *Cx. tarsalis* (R^2=0.09, F ratio=2.90, df=31, P=0.0991). The second set of analyses, which treated site visits as replicates and included mosquito abundances for individual site visits as response variables, indicated that abundance of *Ae. albopictus* increased with increasing ERC cover and varied among site visits, with more mosquitoes collected later in the season (Table 2). This analysis also indicated that the mean abundance of *Ps. columbiae* and *An. quadrimaculatus* was inversely related to ERC cover with abundance peak within the sampling season (*Ps. columbiae*) or later in the season (*An. quadrimaculatus*)

Table 2. Results of linear regression analyses testing the effects of percent cover of eastern redcedar and site visit on abundance of *Ae. albopictus*, *Ps. columbiae*, *An. quadrimaculatus*, *Cx. tarsalis*, *Cx. erraticus*, and *Cx. pipiens* collected during individual visits to each site.

Variable	Ae. albopictus			Ps. columbiae			An. quadrimaculatus			Cx. tarsalis			Cx. erraticus			Cx. pipiens		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Site visit	5.89	5	**0.0001**	16.72	5	**<0.0001**	9.44	5	**<0.0001**	10.58	5	**<0.0001**	10.49	5	**<0.0001**	1.27	5	0.2780
ERC percentage	14.54	1	**0.0002**	32.66	1	**<0.0001**	4.65	1	**0.0324**	2.37	1	0.1302	1.44	1	0.2319	0.55	1	0.4577

(Table 2). Although abundance increased later in the season for *Cx. tarsalis* and *Cx. erraticus*, this analysis again found no significant effects of ERC cover for these species, and there were no significant effects of either site visit or ERC cover for *Cx. pipiens* (Table 2).

West Nile virus results

Of a total of 111 pools tested for WNV (44 pools of *Cx. pipiens* and 67 pools of *Cx. tarsalis*), only one pool of *Cx. tarsalis* tested positive. We therefore could not conduct formal analyses evaluating the relationship between ERC encroachment levels and WNV infection in mosquitoes. The single positive pool consisted of 18 *Cx. tarsalis* collected in a zone 3 site with 26.04% ERC cover near Binger (Caddo County) on September 19, 2020.

DISCUSSION

Our study illustrates that ERC encroachment is affecting mosquito abundance across a geographically diverse area in the southern U.S. Great Plains. Across four sampled areas in central and western Oklahoma, abundance of *Ae. albopictus* was positively related to percent cover of ERC, while *Ps. columbiae* abundance decreased with increasing ERC cover. Because this study occurred in a year with relatively low levels of WNV transmission, we were unable to formally evaluate the association between ERC encroachment and WNV prevalence in mosquitoes. Nevertheless, the only WNV-infected pool of mosquitoes (*Cx. tarsalis*) was collected in an ERC tree. Furthermore, due to sampling limitations described below, we cannot entirely rule out that ERC encroachment widely increases abundance of other medically important mosquito species, such as *Cx. tarsalis*.

Previous studies found higher abundances of *Culex pipiens* and *Cx. tarsalis* in areas with ERC encroachment compared to open grasslands (O'Brien and Reiskind 2013, Noden et al. 2021a), but these studies did not evaluate how mosquito populations change with varying levels of ERC encroachment. Such an analysis is critical for understanding relationships between ERC, mosquito populations, and pathogen-infected mosquitoes, because levels of ERC encroachment are spatiotemporally variable in the Great Plains region (Zou et al. 2018) and because there may be thresholds of encroachment beyond which mosquito populations and WNV transmission are especially impacted. Notably, our analysis using a continuous ERC cover variable suggests that such thresholds may not exist, at least for *Ae. albopictus* and *Ps. columbiae*; instead, mosquito abundances may change continuously with increasing ERC encroachment. If abundances of important mosquito species involved in transmission of human and animal pathogens increase as levels of ERC cover increase, it may be possible to reduce the risk of exposure to mosquito-borne pathogens by reducing or entirely removing ERC cover. However, further research is needed to determine the scale of ERC management (e.g., tree cover reduction or complete removal of all trees from individual properties vs across a larger landscape) that results in substantial reductions in health risk on both individual properties and in larger

jurisdictions (e.g., at the county level). Nonetheless, this study provides unprecedented information about effects of ERC on mosquito communities by demonstrating that abundance of adult host-seeking *Ae. albopictus* increases, and *Ps. columbiae* and *An. quadrimaculatus* abundance decreases, with increasing ERC encroachment.

Aedes albopictus was first detected in Oklahoma in 1990 and spread throughout the state by 2004 (Noden et al. 2015). The principal vector species of canine heartworm in the state (Paras et al. 2014), *Aedes albopictus* is also a bridge vector for WNV (Rochlin et al. 2019) and a competent vector for multiple human (yellow fever, dengue, Chikungunya, and Zika) and animal viruses (eastern equine encephalitis, western equine encephalitis) (Miller and Ballinger 1988, Scott et al. 1990, Beaman and Turell 1991, Mitchell 1991, Licitra et al. 2010). This species is primarily associated with urban areas (Paras et al. 2014, Bradt et al. 2019, Sanders et al. 2020) but in the present study, collections were made in rural areas where its abundance is relatively low (Noden et al. 2021a). Given the widespread encroachment of ERC into urban and suburban areas in the region (Coppedge et al. 2001a), our finding of a positive relationship between ERC cover and *Ae. albopictus* abundance has critical implications for human health in cities. Future studies should therefore evaluate effects of urban ERC encroachment on the nidi of infection for the many established and emerging human arboviruses vectored by this mosquito species (Thomas et al. 2016, Adams et al. 2019).

Abundance of *Culex tarsalis*, the main vector for WNV in western Oklahoma (Noden et al. 2015), was not significantly related to ERC cover in our study, but *Cx. tarsalis* abundance did change significantly across site visits, with 80% of individuals collected during the single round of site visits in September (Table 2). Due to logistical constraints related to the academic calendar, we were limited to sampling at only one zone during each weekend in September. As a result, and as evidenced by 63% of all individuals being sampled in zones 2 and 3, we were unable to capture the relatively brief seasonal peak in *Cx. tarsalis* abundance across all four zones in our study area. Due to this limitation, we cannot entirely rule out that ERC encroachment widely increases *Cx. tarsalis* abundance across central and western Oklahoma, especially since previous studies provide evidence for this effect (Noden et al. 2021a). In future studies, sampling should be conducted with sufficient frequency and spatial coverage to adequately capture abundance peaks for *Cx. tarsalis* to allow rigorous tests of the effects ERC encroachment has on this species.

Culex tarsalis is a strong flier that feeds on a diverse array of mammals and birds and is the most important mosquito involved in WNV transmission in the U.S. from the Great Plains to California (Kent et al. 2009, Dunphy et al. 2019). Even though WNV prevalence and detection has been relatively low in Oklahoma since 2018, this is the third study since 2017 that has collected WNV-infected *Culex* in ERC-encroached areas of Oklahoma (Hess et al. 2020, Noden et. al 2021a). While there was no relationship between *Cx. pipiens* abundance and ERC cover in the current study, this species has also previously been found to be more abundant in ERC than

open grassland in central Oklahoma (Noden et al. 2021a). The connection of ERC with known *Culex* vector species in the southern Great Plains suggests this encroaching tree poses a risk for local infections and outbreaks of Cache Valley virus, St. Louis encephalitis virus (Curren et al. 2018), western equine encephalitis virus (Reeves et al. 1947, Randolph et al. 1994), and eastern equine encephalitis (Lindsey et al. 2018b).

A previous study linked high levels of *Ps. columbiae* abundance with grassland habitat instead of ERC and other tree species (Noden et al. 2021a). The current study further demonstrates that *Ps. columbiae* occurs in highest numbers in open grasslands and that their abundance declines with increasing ERC cover. Primarily considered a nuisance floodwater mosquito for cattle and horses (Kuntz et al. 1982), *Ps. columbiae* is a possible minor vector of WNV but is not considered to contribute to human outbreaks (Godsey et al. 2012). This species also has shown potential to transmit Venezuelan equine encephalitis virus, Rift Valley fever virus, and canine heartworm (Moncayo et al. 2008, Paras et al. 2014, Turell et al. 2015). The abundance of *Anopheles quadrimaculatus*, the historical vector for human malaria in the region, was also highest in grassland habitats and declined with increasing ERC cover. Overall, results from this study agree with others that indicate individual mosquito species have preferences for habitats that may facilitate their transmission of pathogens (Reiskind et al. 2017). The relationships we document here demonstrate the importance of identifying effects of encroachment by ERC and other woody species on specific vector species, as this species-specific information will improve understanding of which vector-borne disease systems are likely to be impacted by encroachment.

No study is without limitations, and this study was limited by several challenges beyond our control. The greatest challenge we encountered was the onset of the SARS-CoV-19 pandemic in March, 2020. Specifically, national and university COVID-19 lockdown protocols delayed field data collection in 2020, as we could not initially establish collection sites, directly interact with landowners regarding access to their property, or stay in hotels in our study area. As mentioned above, sampling was further constrained by the academic calendar; the beginning of classes in August, 2020 required us to shift the sampling interval at each site from bi-weekly to tri-weekly, thus decreasing sampling frequency during August and September, when *Culex* sp. numbers peak in our study region (Noden et al. 2021a). Despite these challenges, we collected enough mosquitoes throughout the study period to analyze relationships between mosquito populations and varying levels of ERC encroachment. However, future studies that more intensively sample across the entire season of mosquito activity, and across greater than one year, are needed to further elucidate effects of ERC on mosquitoes and mosquito-borne pathogen transmission.

In conclusion, results from this study suggest that WPE could increase the transmission of mosquito-borne pathogens, particularly West Nile virus, in the southern Great Plains of the U.S. Mechanisms underlying any potential effects on WNV are uncertain and require additional

research; however, they could include provision of food (e.g., fruit (Reiskind and Zarrabi 2011) and host blood meals) and shelter for resting mosquitoes (Cote, unpublished data) and abiotic conditions that reduce desiccation and increase survival and/or reproduction in an otherwise arid landscape (Loss et al. 2022). ERC also provides habitat for some bird and mammal species that provide mosquito blood meals (Coppedge et al. 2001a, Horncastle et al. 2004, Reddin and Krementz 2016), including the American robin (*Turdus migratorius*), a key WNV reservoir and "superspreader" in other regions (Hamer et al. 2009, Kilpatrick et al. 2006). Yet, the relative importance of different bird species as mosquito blood meal hosts and WNV amplifiers in ecosystems experiencing ERC encroachment has not been evaluated and requires further research. Notably, ERC encroachment also appears to be facilitating expansion of tick-borne diseases in the Great Plains region due to provision of a relatively humid microhabitat that allows ticks to invade previously inhospitable areas (Noden and Dubie 2017) and shelter for reservoir hosts (white-tailed deer; *Odocoileus virginiana*) that are key to transmission of human tick pathogens (*Ehrlichia* species) (Yabsley 2010). ERC may also provide habitat for horseflies that can vector pathogens (e.g., *Anaplasma marginale*) that affect livestock, and thus agricultural production, in this economically important region. Together, these examples illustrate the need for major research advances to improve understanding, prediction, and management of WPE effects on vector-borne disease transmission in the U.S. Great Plains and beyond.

Acknowledgments

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