Scientific Note

Involvement of invasive eastern red cedar (*Juniperus virginiana*) in the expansion of *Amblyomma americanum* in Oklahoma

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Tick-borne diseases remain the most important vector-borne diseases in the United States (CDC 2016). While Lyme disease continues to be the most important, others, including spotted fever group (SFG) rickettsiosis (historically Rocky Mountain spotted fever), ehrlichiosis, anaplasmosis, and a number of emerging pathogens, are regionally impacting public and veterinary health (Pritt et al. 2011, Krause et al. 2013, Savage et al. 2013, Kosoy et al. 2015, Pritt et al. 2016). Since the early 1980s, Oklahoma has had one of the highest incidence rates for SFG rickettsiosis as well as ehrlichiosis caused by *Ehrlichia chaffeensis* and/or *Ehrlichia ewingii* (Dalton et al. 1995, Treadwell et al. 2000, Chapman et al. 2006, Openshaw et al. 2010, Dahlgren et al. 2011). Added to this list, Oklahoma is currently third in the U.S. for incidence of tularemia (CDC 2013) along with the third fatal case of Heartland virus in July, 2014 and the second case of Bourbon virus in May, 2015.

Reports of the incidence of SFG rickettsiosis at the county level within the United States have demonstrated SFG rickettsiosis at greater than 60 cases/1,000,000 persons/year almost throughout the entire state of Oklahoma (Biggs et al. 2016). While on a lesser scale, similar results are observed for ehrlichiosis (Heitman et al. 2016). Limited focus, however, has been given to changing epidemiological patterns within the state of Oklahoma over the last two decades. The changing epidemiology of these diseases suggests the probability of an expanding tick vector which has recently spread throughout the state. The distribution of *Dermacentor variabilis*, the main vector for *R. rickettsii* (Rocky Mountain spotted fever), has been relatively static since 1940 (Mitcham et al. 2017) but populations of *Amblyomma americanum*, the main vector for *R. amblyommii*, *E. chaffeensis*, and *E. ewingii*, have shifted dramatically westward since the 1970s (Barrett et al. 2015). The potential involvement of *A. americanum* in the geographical increase of these important tick-borne diseases (Paddock and Yabsley 2007, Dahlgren et al. 2016) generates important ecological questions as to how a tick species, normally associated with more humid environments, has been able to establish viable populations in dry, arid western Oklahoma.

Since the early 1940s, eastern red cedar (*Juniperus virginiana*) has been increasingly invading prairies and mixed woodlands in the Great Plains region (Engle et al. 2008, Meneguzzo and Liknes 2015). Used as windbreaks and shelterbelts across the Great Plains, this plant has rapidly expanded into historic prairies (Engle et al. 2008), traditional oak and hickory forests, (Van Els et al. 2010), and urban areas (Coppedge et al. 2001), dramatically altering the soil and water composition (Pierce and Reich 2010) as well as bird and mammal fauna (Coppedge et al. 2001, Horncastle et al. 2004). One of the major invasion zones for eastern red cedar has been in the southern Great Plains in Oklahoma (Bidwell et al 2000). The aim of the study was to determine whether this invasive plant could be providing a suitable habitat for the western expansion of *A. americanum* on the Great Plains. We hypothesized that eastern red cedar is not only an important habitat for *A. americanum* in central Oklahoma but also provides adequate habitat for lone star ticks (*A. americanum*) to establish in western Oklahoma.

Seven locations were chosen throughout central and western Oklahoma: four sites in central Oklahoma (Oklahoma State University (OSU) cross-country track and OSU range research pasture near Stillwater, private pasture in Mulhall, and private farm in Norman) and three sites in western Oklahoma (Roman Nose State Park, American Eagle Lake Recreation Area, and the OSU Marvin Klemme Range Research Station, Bessie, OK) (Figure 1). These representative sites were close to sites already being sampled by the authors or they were discovered using Google Earth (version 7.1.7.2606, Microsoft). Sites were characterized by differing stages, estimated by height and location (Engle and Kulbeth 1992), of invading eastern red cedar: young (4-6 years old), medium (6-12 years old), and mature forest (12+ years old) and mixed forest containing oak and hickory (Figure 2). Between April and June, 2015, sites were visited once and ticks were collected for one-hour periods between 09:00 and 12:00 using five to nine CO_2 traps described in Barrett et al. (2015). This period of the year was chosen because it is when *A. americanum* ticks are most active in Oklahoma and thus, most likely to be collected using CO_2 traps (Barrett et al. 2015). This method was chosen as the most effective for collecting *A. americanum* within a three to five m radius. Each CO_2 trap in a given area was placed close to the trunk of a tree and within red cedar thickets 20 m from other CO_2 traps to ensure that most of the habitat being surveyed primarily consisted of eastern red cedar. All collected ticks were stored in 70% EtOH and identified in the laboratory using standard keys (Keirans and Litwak 1989).

A total of 708 *A. americanum* (n=584; 82%) and *D. variabilis* (n=124; 18%) was collected among all eastern red cedar sampling sites across the region (Table 1). A majority (91.4%) of *A. americanum* collected in the study were from sites in central Oklahoma compared with only 45.2% (n=56) of the *D. variabilis*. Differing levels of tick infestation were apparent among sites with most ticks collected at the Mulhall and Norman sites and the least in Roman Nose State Park. *Amblyomma americanum* greatly outnumbered *D. variabilis* in all sites in central Oklahoma while *D. variabilis* was the more common species collected in all western sites. There were significant differences of *A. americanum* across varying levels of red cedar maturity (Kruskall-Wallis: 10.493, df=4,

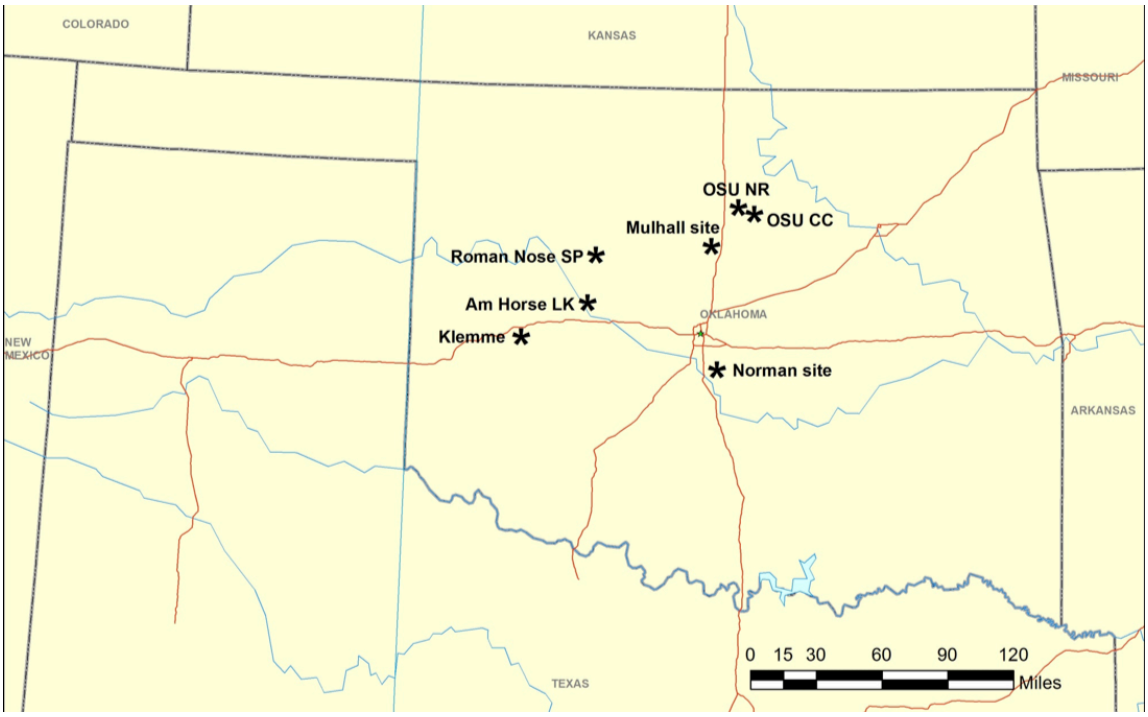

Figure 1. Tick collection sites in eastern red cedar stands across central and western Oklahoma, April to June, 2015. Sites include: Oklahoma State University north research range (OSU NR) and cross-country track (OSU CC); Mulhall pasture sites, Norman farm site, Roman Nose State Park (SP), American Horse Lake Recreation area (Am Horse LK), and the OSU Marvin Klemme Range Research Experiment Station (Klemme).

p>0.033) with a higher median of lone star ticks in mixed forests (median=28; range 9-52) as well as in young (median=5; range 0-87) and medium-aged (median=5; 0-51) red cedar stands. In the Mulhall site, 95% of the *A. americanum* collected were among the young cedar stands while 4% were collected in the old forest and 1% in the area with no eastern red cedar.

These results appear to demonstrate that *A. americanum* use eastern red cedar of varying maturity levels as habitat in central and western Oklahoma. As the majority species of tick collected in a variety of habitats throughout the central region, it is apparent that *A. americanum* has successfully invaded this area (Barrett et al. 2015). Based on records from passive surveillance using submissions to the K.C. Emerson Entomology Museum (OSU Department of Entomology and Plant Pathology), and the OSU Plant Disease and Insect Diagnostic Lab, and discussions with local residents, *A. americanum* became broadly established in central Oklahoma at the end of the 1980s with most samples submitted after 1990 (Grantham, unpublished data). Since this period, *A. americanum* has become established as the main tick species in the region, effectively using all habitats, including the oak-hickory dominated cross-timbers habitat as well as the areas with invasive eastern red cedar.

The invasion of *A. americanum* into the western side of Oklahoma (west of Oklahoma City), however, appears to be a rather recent event. An area characterized as dry and arid, the low numbers of *A. americanum* recovered as compared with *D. variabilis* were notable. While eastern red cedar may be assisting in the establishment of the species in the region, lone star ticks have still not adapted to the new environmental conditions. Conditions in western Oklahoma can be harsh with frequent droughts, but the invasive red cedar tends to be found in gullies and lower areas that are normally associated with moister soils. These habitats are well suited for shelter for the animals which act as reservoirs and transporters of ticks, including deer, turkeys, and coyotes as well as cattle which use eastern red cedar to escape the heat (O'Brien and Reiskind 2013, Masters et al. 2014). Hunters are exposed to ticks while walking or waiting near red cedars, while ranchers are exposed due to occupational activities such as driving cattle or looking for lost calves in the red cedar areas (Noden, personal observation).

This expansion of *A. americanum* across the state may be linked to the increased expansion and movement of white-tailed deer into the central and western areas of Oklahoma since the 1970s (QDMA 2009). White-tailed deer are considered the main mammal involved in the life cycle and distribution of *A. americanum* (Paddock and Yabsley 2007) as well as four other important species of ticks found in Oklahoma: American dog tick (*D. variabilis*), Gulf Coast ticks (*Amblyomma maculatum*), Blacklegged ticks (*Ixodes scapularis*), and Winter ticks (*Dermacentor albipictus*). Since the virtual extinction of white-tailed deer in Oklahoma in the 1930s, numbers of harvested deer have steadily increased over time. By the 1980s, the numbers of annual harvested deer in Oklahoma was around 180,000, rising to over 500,000 in recent years (QDMA 2009). This growth has meant an increase in white-tailed deer searching for new territory and subsequent movement of the ticks feeding on them. The increasing expansion and population growth of white-tailed deer into central and western counties also corresponds with

Table 1. Breakdown of tick species by red cedar type collected at seven eastern sites across central and western Oklahoma, April to June, 2015.

Area of Oklahoma	Collection Date	Sampling Site	Red Cedar category	# of CO_2 traps	Tick Species	Total ticks collected	Proportion Aa / site*	Average ticks/trap	Range/trap (median)
Central	April 3, 2015	OSU Cross-Country (OSU CC)	Medium	5	*A. americanum*	27	96.4	5	3-8 (6)
					D. variabilis	1		0	0-1 (0)
	April 1, 2015	OSU Research Range (OSU RR)	Mixed	5	*A. americanum*	161	98.2	32	16-52 (34)
					D. variabilis	3		1	0-3 (0)
	April 9, 2015	Mulhall	Young	5	*A. americanum*	159	98.1	32	0-87 (22)
					D. variabilis	3		1	0-1 (1)
			Old	4	*A. americanum*	18	100	5	1-11 (4)
					D. variabilis	0		0	0 (0)
			No cedar	4	*A. americanum*	4	100	1	(1-4 (1)
					D. variabilis	0		0	0 (0)
	April 21, 2015	Norman	Medium	7	*A. americanum*	178	80.5	25	10-51 (24)
					D. variabilis	43		6	0-33 (2)
			Mixed	1	*A. americanum*	9	75.0	9	9 (9)
					D. variabilis	3		3	3 (3)
			No cedar	1	*A. americanum*	0	0.0	0	0 (0)
					D. variabilis	1		1	1 (1)
West	June 10, 2015	American Horse Lake (Am Horse LK)	Young	3	*A. americanum*	0	0.0	0	0 (0)
					D. variabilis	6		2	0-4 (2)
			Medium	3	*A. americanum*	0	0.0	0	0 (0)
					D. variabilis	6		2	2 (2)
	June 10, 2015	Roman Nose State Park (Roman Nose SP)	Medium	5	*A. americanum*	1	33.3	0	0-1 (0)
					D. variabilis	2		0	0-1 (0)
	May 11, 2015	Klemme Range Research Station	Young	6	*A. americanum*	49	47.6	8	0-19 (7)
					D. variabilis	54		9	0-30 (6)

* Proportion of *Amblyomma americanum* vs *Dermacentor variabilis* at each trap site.

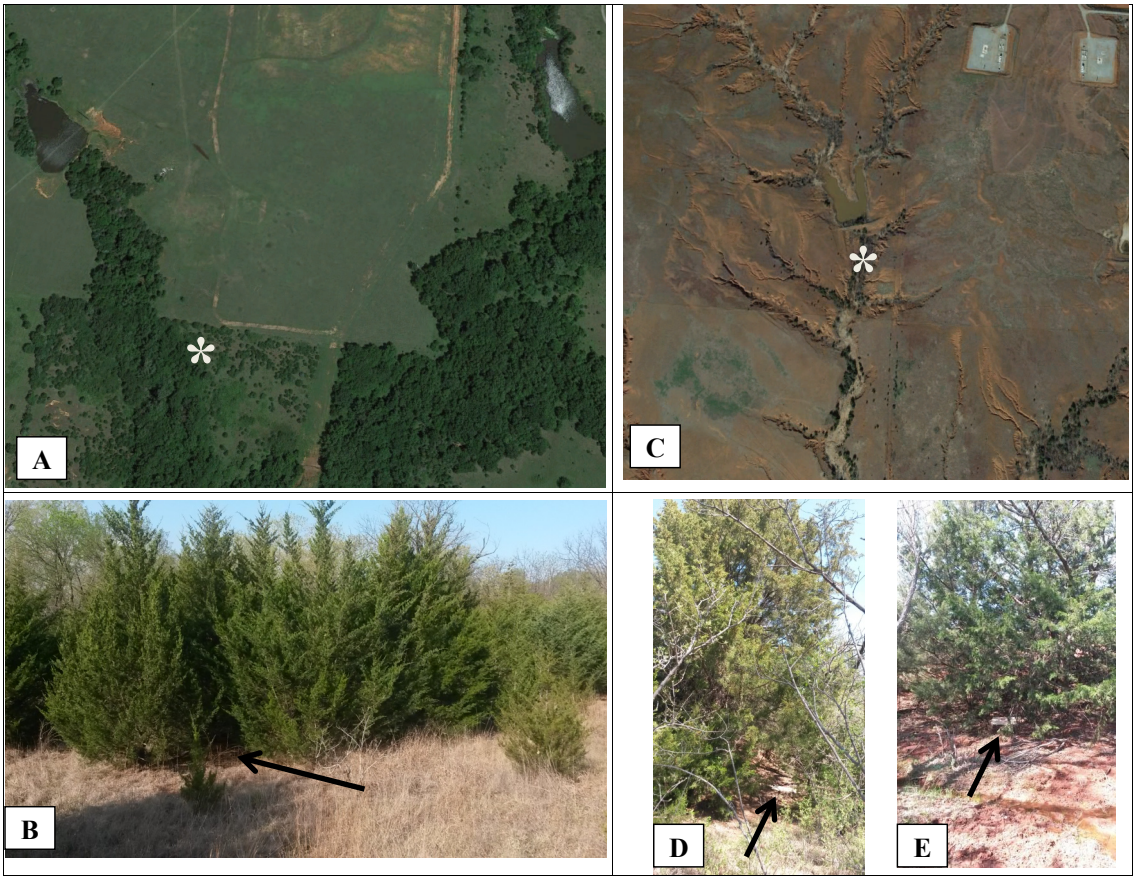

Figure 2. Two sampling areas (white stars) in eastern red cedar stands in A) central (Mulhall pasture site) and C) western (Klemme Experimental Station) Oklahoma. Examples of varying red cedar maturity and positioning of CO_2 traps (arrows) where ticks were collected in central (B) and western (D and E) Oklahoma.

an increase of deer within urban areas across the state. A recent study in Oklahoma City collected over 500 ticks in eight urban sites, several of which were completely surrounded by developed land, yet established populations of *A. americanum* were present at most of the sites (Noden et al. 2016).

The proportions of *A. americanum* compared with *D. variabilis* were notably different between central and western Oklahoma sites (Table 1). The competitive displacement of *D. variabilis* or other tick species by *A. americanum*, also known as the 'Lone Star effect' (Nadolny et al. 2014), has been noted in the literature. Where *A. americanum* have established their populations, it seems that other species, *D. variabilis* (Berrada et al. 2011) in particular, are less abundant (Nadolny et al. 2014). This trend can be observed in our data from central Oklahoma sites where *A. americanum* were between 79% and 98% of the ticks recovered. The broad feeding habits of this species, in which all stages will feed on any blood source, makes it more able to invade new habitats where ticks with more narrow feeding preferences are found (Paddock and Yabsley 2007). The variation in ratios between these two species from western Oklahoma sites provides an opportunity to monitor competitive relationships between *A. americanum* and *D. variabilis* within in a given area over time.

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