RESEARCH ARTICLE

H sA N E P T P s S s U S s

. . F [1]*, . E. [2], . D. C [3], . [4], . a [5], . . a [6], A. O [7], . [8], A. E. [5]

1 Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, Tennessee, United States of America, **2** Department of Biology, Christian Brothers University, 650 East Parkway South, Memphis, Tennessee, United States of America, **3** Department of Biology, Rhodes College, 2000 North Parkway, Memphis, Tennessee, United States of America, **4** Department of Earth Sciences, University of Memphis, Memphis, Tennessee, United States of America, **5** Department of Forestry, Wildlife and Fisheries, University of Tennessee, Knoxville, Tennessee, United States of America, **6** Department of Biosystems Engineering and Soil Science, University of Tennessee, Knoxville, Tennessee, United States of America, **7** Department of Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, Tennessee, United States of America, **8** Department of Biology, University of Memphis, Memphis, Tennessee, United States of America

* RFryxell@utk.edu

ssMark
click for . ates

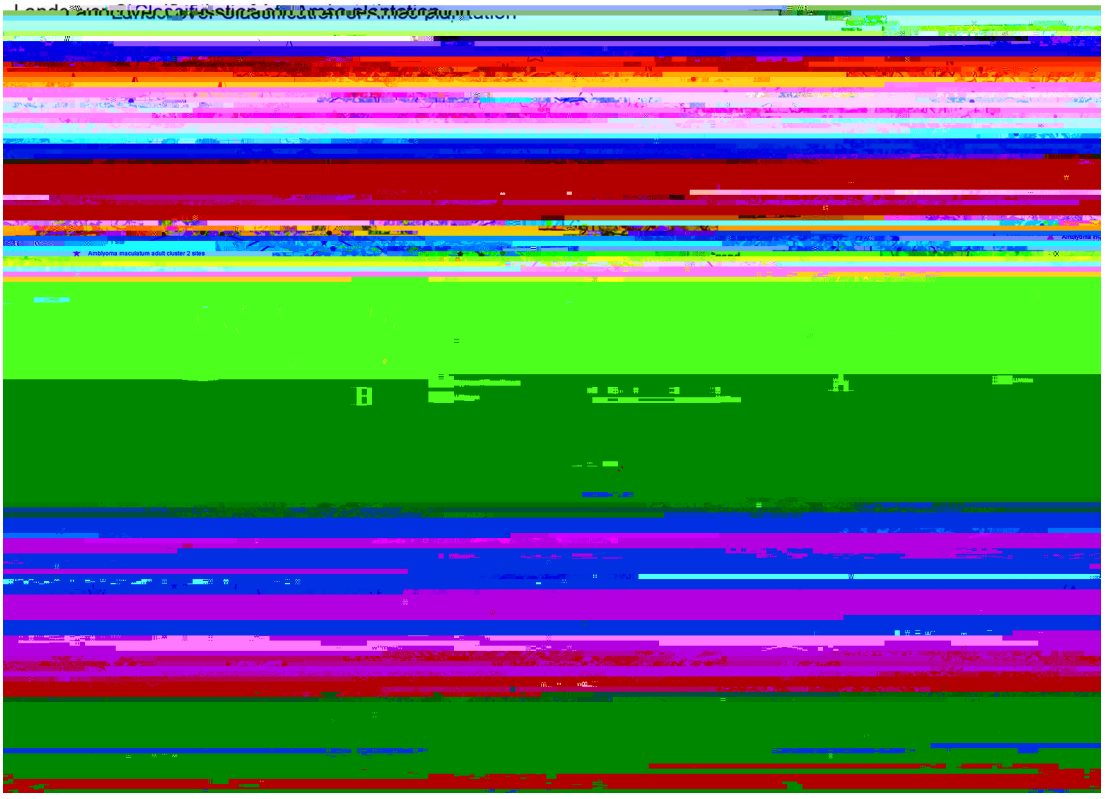
A s

Two tick-borne diseases with expanding case and vector distributions are ehrlichiosis (transmitted by *Amblyomma americanum*) and rickettiosis (transmitted by *A*. *maculatum* and *Dermacentor variabilis*). There is a critical need to identify the specific habitats where each of these species is likely to be encountered to classify and pinpoint risk areas. Consequently, an in-depth tick prevalence study was conducted on the dominant ticks in the southeast. Vegetation, soil, and remote sensing data were used to test the hypothesis that habitat and vegetation variables can predict tick abundances. No variables were significant predictors of *A*. *americanum* adult and nymph tick abundance, and no clustering was evident because this species was found throughout the study area. For *A*. *maculatum* adult tick abundance was predicted by NDVI and by the interaction between habitat type and plant diversity; two significant population clusters were identified in a heterogeneous area suitable for quail habitat. For *D*. *variabilis* no environmental variables were significant predictors of adult abundance; however, *D*. *variabilis* collections clustered in three significant areas best described as agriculture areas with defined edges. This study identified few landscape and vegetation variables associated with tick presence. While some variables were significantly associated with tick populations, the amount of explained variation was not useful for predicting reliably where ticks occur; consequently, additional research that includes multiple sampling seasons and locations throughout the southeast are warranted. This low amount of explained variation may also be due to the use of hosts for dispersal, and potentially to other abiotic and biotic variables. Host species play a large role in the establishment, maintenance, and dispersal of a tick species, as well as the maintenance of disease cycles, dispersal to new areas, and identification of risk areas.

The roles of *Am mm m um* (lone star tick), *Am mm m u um* (Gulf coast tick), and *m* (American dog tick) in tick-borne disease (TBD) transmission has been directed at host association studies [1–6], but field studies investigating habitat

overlying sedimentary rock [28], and these data have been used to identify potential locations with Lyme disease (aka risk areas). Thus, the habitat's microclimate, vegetation, and soil type likely have significant effects on tick abundance and questing activity and on the dynamics of TBDs. Habitat suitability also includes other stages in a tick's life, including overwintering, molting, and oviposition sites.

Previous work at Ames Plantation in southwestern Tennessee and within the previously



F 1. a a a a (A)a a a a a Amblyomma maculatum a a a **(B),** Dermacentor variabilis a a a **(C)** a Ixodes scapularis a a **(D).** Land cover classification is generated from the Landsat 8 OLI image downloaded at http://earthobservatory.nasa.gov

:10.1371/ . .0144092. 001

Tick Collection

Based upon a trapping methods comparison ( ), CO_2-dry ice traps, conventional dragging, CO_2-dragging, and CO_2-flagging were used for tick sampling during June of 2014 when tick-borne diseases peak in Tennessee [7, 33, 34]. One dry ice trap was set at the middle of each center 100m transect and left overnight. The remaining three collection methods were randomly assigned to each 100m transect and checked for ticks every 20m along each transect. All encountered ticks were removed, counted, and placed in vials of 80% ethanol. Ticks were identified in the laboratory to life-stage, species, and sex [35–37].

Vegetation Characterization

Vegetation was sampled in a $1m^2$ quadrat at the center point of each tick drag along the center transect at each site. Therefore, plant community composition and structure were sampled at 10, 30, 50, 70, and 90m along each centerline. Within each sampling area, each plant species was identified and percent cover of each species was visually estimated with an adapted Daubenmire cover scale (<1%, 1–5%, 5–10%, 10–25%, 25–50%, 50–75%, 75–95%, > 95%) and transformed to median values for community similarity analyses. Transect data for each site were combined for both diversity and composition pattern analyses. To determine percentage canopy openness and leaf area index (LAI), hemispherical photographs taken in the transect center (50m) with a fisheye lens on a 1m tripod were analyzed with Gap Light Analyzer software [38]. All photographs were taken on cloudless days in late May between 0830 and 1330 h.

To determine vertical structure, basal area was estimated using a handheld prism, identifying and including tree species with a diameter at breast height (dbh) $>$ 5cm at each sampling point

sites (*A. m* *m* nymphs and adults), MANOVA was used to examine habitat use. For MANOVAs, the dependent variable was the number of individuals in each stage class (adults or nymphs) at a site. For tick species in which only adults were well represented (*A. m* *m* and .), an ANOVA was used with number of adult ticks at a site as the dependent variable. Dependent variables were log-transformed to meet model assumptions. In both MANOVA and ANOVA, our predictor variables were habitat type, plant diversity (Shannon Index), plant evenness (E_h

upland deciduous, 2.1 ± 0.58 in coniferous, and 0.8 ± 0.28 in grassland sites. The presence of . did not differ across habitats (^{2} = 1.32; df = 3; = 0.725), and neither did population sizes (= 0.4724; df = 3, 72; = 0.7025).

Comparisons of $A.$ m life stages mirrored total $A.$ m collections. A total of 727 adults were collected of which significantly more were collected at coniferous sites (15.9 ± 2.12) and upland deciduous sites (12.8 ± 2.79) than bottomland deciduous sites (5.1 ± 1.30) and grassland sites (3.0 ± 0.72) (= 12.34; df = 3, 72; $<$

a 2. E a a A E a 0.7 (a a 0.62 a -

	A	B D	a /	C	D	a / a	a	*E C
Agriculture	21	3	4	0	1	1	30	0.30
Bottomland Deciduous	3	17	2	4	7	0	33	0.48
Grass/pasture	1	1	14	0	0	1	17	0.17
Coniferous	1	2	0	20	4	1	28	0.28
Upland Deciduous	1	5	1	3	22	0	32	0.31
Water/marsh	0	0	1	0	0	19	20	0.05
Total	27	28	22	27	34	22	160	
**Error of Omission	0.22	0.39	0.36	0.25	0.35	0.13		0.70

*Error of commission: percentage of misclassified pixel against ground truth data

**Error of omission: percentage of omitted pixel against ground truth data

:10.1371/ . .0144092. 002

of the total Sum of Squares (Table 4). For . , no variables were significant predictors of adult tick abundance (Table 5).

Cluster Detection and Identification

Spatial clusters were not identified for *A. m* *m* total counts, adult or nymphal counts indicating the species and life stages were found throughout Ames Plantation (> 0.05).

a 3. A O Aa a a Amblyomma americanum a a a a a a a .

a	D	a	A a	F a	. D	D . D	P a
habitat type	3	0.133553	0.8348		6	70	0.54717

a . 4. A O Aa . a . a Amblyomma maculatum a . a A. maculatum a . D - a . -
a a a a a a a .

D		a	F a .	P a .
habitat type	3	0.164	0.0547	0.721	0.5463
plant diversity	1	0.0263	0.0263	0.346	0.5601
plant evenness	1	0.1009	0.1009	1.331	0.2565
basal area	1	0.1694	0.1694	2.234	0.1439
distance to roads	1	0.0101	0.0101	0.133	0.718
patchiness	1	0.006	0.006	0.079	0.7804
openness	1	0.0005	0.0005	0.007	0.9336
D -	1	0.3844	0.3844	5.068	**0.0308***
soil pH	1	0.0041	0.0041	0.054	0.8172
water	1	0.0056	0.0056	0.074	0.7868
a . a : a	3	0.853	0.2843	3.749	**0.0195***
habitat type: plant evenness	3	0.1389	0.0463	0.611	0.6126
habitat type: basal area	3	0.0265	0.0088	0.117	0.9498
habitat type: distance to roads	3	0.3715	0.1238	1.633	0.1994
habitat type: patchiness	3	0.0427	0.0142	0.188	0.9041
habitat type: openness	3	0.4738	0.1579	2.082	0.1203
habitat type: NDVI	3	0.0119	0.004	0.053	0.9839
habitat type: soil pH	3	0.1233	0.0411	0.542	0.6568
habitat type: water	3	0.1516	0.0505	0.666	0.5784
Residuals	35	2.6543	0.0758		

B . a . a a (* P < 0.05).

:10.1371/ . .0144092. 004

Analysis of $A.\ m$ m adults identified two statistically significant clusters, one cluster with 1 site (= 64.6, = 0.05) and a second cluster with sixteen sites (= 7.3; = 0.028) (Fig 1b). Analysis of . adults identified three statistically significant clusters, one cluster with five sites (= 3.1, = 0.0005), a second cluster with one site (= 6.8, = 0.0016), and a third cluster with four sites (= 3.1; = 0.029) (Fig 1). While only seven . were collected from four sites, these seven were found in a single statistically significant cluster (= 80.0, = 0.001) (Fig 1d).

D .

A thorough understanding of tick populations and their pathogens is essential to the accurate and timely diagnosis of TBDs, the development of risk assessments, and advancement of management plans to control ticks and reduce TBDs. This study identified few vegetation and landscape variables associated with tick presence or density, and this is likely due to ticks using hosts for dispersal and limiting our environmental variables to vegetation and landscape features to one tick season and study site. This study focused on vegetative and landscape features because these data are easier to obtain over broad geographic regions and because these traits likely influence ticks directly through abiotic factors and indirectly through their effects on host species. While some variables, such as NDVI and the interaction between habitat type and plant diversity, were significantly associated with one tick species, the amount of variation explained was low and not useful for predicting presence or density reliably. The NDVI coefficient was extremely small ($9x10^{-14}$) and likely an artifact of the large number of 0s (tick absence); while statistically significant, the association is weak and not biologically useful for

prediction. This low amount of variation explained by our models is likely due to the use of hosts for dispersal [47, 48] and potentially other environmental variables such relative humidity [49, 50] and soil conditions [28]. Host species play a large role in the establishment, maintenance, and dispersal of a tick species, as well as the maintenance of disease cycles and associated pathogens [29, 32, 51]. Identification of preferred host species for each tick species in western Tennessee will help determine the factors that aide in the establishment and maintenance of tick populations and identify potential mechanisms (i.e., host agents) of tick (and pathogen) dispersal.

Although we were unable to identify specific environmental variables associated with each tick species and/or life stage, we found significant spatial clusters for $A.\ m$ u um, . -, and . u . Again, we speculate that this clustering might be due to 1) uncharacterized environmental variables, 2) the need for additional seasonal sampling and replication, and 3) the use of hosts for dispersal. Other constraints that might favor or limit tick populations include the assemblage of host species and habitat parameters. This might include not just using hosts for dispersal and a food source, but also for "directed dispersal",

agriculture with crops, field, and woods, but all with generally hard, clearly defined edges. For a cosmopolitan tick such as .

habitats. Mesomammals such as raccoons, skunks, and opossums can also be found throughout the plantation, and these species have a generalized affinity for habitats, and there are likely some occasional seasonal tendencies, but few will be predictable. Ticks using these animals as primary hosts will probably be found in mixed environments, and true predictability with repeatability will be rare. Hosts with small home ranges, such as birds and small mammals, will likely have the greatest impact on tick populations. These animals commonly prefer specific vegetation types, and other abiotic/biotic parameters. As a part of the preliminary host studies, white-footed deer mice were collected in field, hardwood, and pine habitats; however, hispid cotton rats were only collected in field grass habitats. Hispid cotton rats are noted as primary hosts for immature *A. m* [29, 55]. This leads us to hypothesize that host-habitat specificity will also likely influence tick presence and absence.

Although large numbers of ticks were collected, a majority of the questing ticks were *A. m*. The critical next step is to determine 1) how the environment influences a tick's ability to locate a host, 2) how the environment influences the presence and abundance of potential tick hosts, and 3) how environmental variables and host community jointly influence population sizes and dispersal of each tick species (and subsequent pathogens). As with and Lyme disease, it is likely that the environment provides shelter and food sources for southeastern ticks with the ability to transmit and/or pathogens. Moreover, the environment provides questing sites for tick attachment, and questing sites are essentially where pathogen transmission begins. Additional drivers into tick range expansion likely include climate warming and/or habitat change as both will affect the plant composition and subsequent host composition for ticks and their pathogens. Further studies into this system that include hosts, vectors, and pathogens [66] that describe the nidus of pathogen transmission [67] such as those presented by Simon et al. [68] are necessary for these southern TBDs.

These data serve as groundwork for commonly encountered ticks and for tick-habitat associations in the southeastern United States and demonstrate a need for 1) continued work on tick-habitat associations that include multiple seasons and sampling efforts, 2) inclusion of hosts in future studies, and 3) concurrent pathogen detection studies to identify areas with pathogen-infected ticks. These findings will assist future endeavors at field sites and serve as foundational data for tick distribution models for the region. Consequently, these findings serve as the basis for determining species distribution, identifying local tick habitats, and analyzing tick biological patterns. With additional tick and pathogen surveillance, these and additional data could contribute t19.8(therurespris0-1.29(and)-2s.)-(plant)-2543on,

12. Calhoun EL. Natural occurrence of tularemia in the Lone star tick, Amblyomma americanum (L), and in dogs in Arkansas. Am J Trop Med Hyg. 1954; 3: 360–366. PMID: 13138839

13. Hopla CE. The transmission of tularemia organisms by ticks in the southern states. Southern Med J. 1960; 53: 92–97. PMID: 14403044

14. Savage HM, Godsey MS Jr, Lambert A, Panella NA, Burkhalter KL, Harmon JR, et al. First detection of heartland virus (Bunyaviridae: Phlebovirus) from field collected arthropods. Am J Trop Med Hyg. 2013; 89: 445–452. doi: 10.4269/ajtmh.13-0209 PMID: 23878186

15. Sumner JW, Durden LA, Goddard J, Stromdahl EY, Clark KL, Reeves WK, et al. Gulf Coast ticks (Amblyomma maculatum) and Rickettsia parkeri, United States. Emerg Infect Dis. 2007; 13: 751–753. PMID: 17553257

16. Burridge MJ, Simmons LA, Peter TF, Mahan SM. Increasing risks of introduction of heartwater onto the American mainland associated with animal movements. Ann NY Acad Sci. 2002; 969: 269–274. PMID: 12381604

17. Price WH. The epidemiology of Rocky Mountain Spotted fever. I. The characterization of strain virulence of *Rickettsia rickettsii*. Am J Hyg. 1956; 58: 248–268.

18. Weiss E. History of rickettsiology Vol 1. *In* Biology of rickettsial diseases. Edited by Walker DH. CRC Press, Inc. Boca Raton FL. 1988. p. 15–32.

19. Stromdahl EY, Hickling GJ. Beyond Lyme: etiology of tick-borne human diseases with emphasis on the southeastern United States. Zoo Public Health. 2012; 59: 48–64.

20. Ginsberg HS, Stafford KC III. Management of ticks and tick-borne diseases In: Goodman J. L., et al. editors. Tick-borne diseases of humans. Washington DC: ASM Press. 2005. p. 65–86.

21.

36. Keirans JE, Litwak TR. Pictorial key to the adults of hard ticks, family Ixodidae (Ixodida: Ixodoidea), east of the Mississippi river. J Med Entomol. 1989; 26: 435–448. PMID: 2795615

37. Keirans JE, Durden LA. Illustrated key to nymphs of the tick genus Amblyomma (Acari: Ixodidae) found

60. Florin DA, Brinkerhoff RJ, Gaff H, Jiang J, Robbins RG, Eickmeyer W, et al. Additional U.S. collections of the Gulf Coast tick, Amblyomma maculatum, (Acari: Ixodidae), from the state of Delaware, the first reported field collections of adult specimens from the state of Maryland, and data regarding this tick from surveillance of migratory songbirds in Maryland. Sys App Acarol. 2014; 19: 257–262.

61. Bishopp FC, Hixon H. Biology and economic importance of the Gulf Coast tick. J Econ Entomol. 1936; 29: 1067–1076.

62. Bishopp FC, Trembley HL. Distribution and hosts of certain North American ticks. J Parasitol. 1945; 31: 1–54.

63. Mather TN, Wilson ML, Moore SI, Ribeiro JMC, Spielman A. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (Borrelia burgdorferi). Am J Clin Epidemiol. 1989; 130: 143–150.

64. Scott JD, Fernando K, Banerjee SN, Durden LA, Byrne SK, Banerjee M, Mann RB, Morshed MG. Birds dispersed ixodid (Acari: Ixodidae) and Borrelia burgdorferi–infected ticks in Canada. J Med Entomol. 2001; 38: 493–500. PMID: 11476328

65. Piesman J, Gern L. Lyme Borreliosis in Europe and North America. Parasitol. 2004; 129: S191–S220.

66. Ogden NH, Tsao JI. Biodiversity and Lyme disease; dilution or amplification? Epidemics. 2009; 1: 196–206. doi: 10.1016/j.epidem.2009.06.002 PMID: 21352766

67. Reisen WK. Landscape epidemiology of vector-borne diseases. Ann Rev Entomol. 2010; 55: 461–483.

68. Simon JA, Marrotte RR, Desrosiers N, Fiset J, Gaitan J, Gonzalez A, et al. Climate change and habitat fragmentation drive the occurrence of Borrelia burgdorferi, the agent of Lyme disease, at northeastern limit of its distribution. Evol App. 2014; 7: 750–764.