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Article

Observations on the seasonality of *Ixodes scapularis* Say in Mississippi, U.S.A.

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Abstract

To assess the seasonality of adult and immature *Ixodes scapularis* ticks, weekly collections were made year-round with a drag cloth at two 0.5 ha sites in northern Mississippi. One hundred and four collection attempts were made between August 1, 2010 and July 31, 2011, and a total of 256 adult and 6 nymphal *I. scapularis* were collected from the sites. From Wall Doxey State Park near the Tennessee state line, 233 adult ticks were collected, whereas only 23 were collected from Noxubee National Wildlife Refuge (NWR), which is located approximately 350 km southeast of that point. Three nymphal *I. scapularis* were collected from each collecting site, and no larval *I. scapularis* were collected at either site. The first adult *I. scapularis* was collected on October 19, 2010 at Wall Doxey Park, while at Noxubee NWR the first adult *I. scapularis* was collected 3 days later (October 21, 2010). The last adults were collected on April 3, 2011 at Noxubee NWR and on May 25, 2011 at Wall Doxey Park. Most adults were caught during March (97 adults, 37.9% of all *I. scapularis* collected), and no adults were collected from June through mid-October. The first *I. scapularis* nymph was collected on August 2, 2010 at Wall Doxey Park. Two more nymphs were collected (one each) at Noxubee NWR on September 3, 2010 and September 16, 2010. Another nymph was collected on March 14, 2011 at Noxubee NWR, then two more on May 17, 2011 at Wall Doxey Park. Statistical analyses showed no significant correlation between vapor pressure deficit (VPD) and number of adult ticks collected, or temperature and VPD combined and number of adult ticks collected.

Key words: *Ixodes scapularis*, sampling, seasonality, Mississippi

Introduction

The blacklegged tick, *Ixodes scapularis* Say, is a North American hematophagous ectoparasite capable of transmitting several pathogens of medical significance to a wide variety of vertebrate hosts, including humans. In the United States, *I. scapularis* is a known vector of *Borrelia burgdorferi*, *Babesia microti* and *Anaplasma phagocytophilum*, the causative agents of Lyme disease, babesiosis, and human granulocytic anaplasmosis, respectively (Wallis *et al*. 1978, Anderson 1989, Dumler 2011, Telford *et al*. 2011). These pathogens are maintained in a sylvatic cycle and humans acquire them via the bite of an infected *I. scapularis*, so to minimize human-tick interaction and exposure to disease agents, it is very important to understand the relative abundance and seasonality of all life stages of this tick species in a given region (Mackay & Foil 2005).

Primarily as a result of Lyme disease, the seasonality and life cycle of *I. scapularis* have been well studied in the northeastern and central U.S.; however, less is known about the biology of this tick in the southern U.S. (Clark *et al.* 2002, Goddard 1992, 1993, Oliver 1993, Mackay & Foil 2005, Goddard & Piesman 2006). For example, in a 10-year survey of human-biting ticks in Mississippi, *I. scapularis* was responsible for 9.2% of reported human tick bites, making it the fourth most

common tick found on humans in Mississippi (Goddard 2002), and in Florida, Georgia and South Carolina, *I. scapularis* was commonly collected in several studies (Felz *et al*. 1996, Cilek & Olson 2000). In Mississippi, there has been limited work describing questing activity of adult *I. scapularis* (Goddard 1986) and immatures (Diuk-Wasser *et al*. 2006, Goddard & Piesman 2006, Diuk-Wasser *et al*. 2010), as well as population estimates of adult *I. scapularis* (Goddard & Goddard 2008) and the relative risk of acquiring these ticks while outdoors (Goddard & Goddard 2010). The objective of this study was to carefully document the questing activity of larval, nymphal, and adult *I. scapularis* in northern Mississippi for an entire year.

Materials and methods

Collection sites. Two 0.5 ha sites in northern Mississippi were selected for sampling, one located at Wall Doxey State Park near Holly Springs (Marshall Co.), and the other at Noxubee National Wildlife Refuge (NWR) near Starkville (Oktibbeha Co.). These locations were chosen because they are wooded with a medium-dense canopy and contain leaf litter and suitable host animals for *I. scapularis*. In addition, both sites had been pre-sampled numerous times to ensure that they contained numerous *I. scapularis* ticks and therefore would be useful for this study. The terrain at Noxubee is lowland, mostly flat with a large, sloping slough covering at least ¼ of the site. For several days following heavy rain, the slough fills with water, preventing complete sampling of the site. The site is located in an area periodically subjected to flooding in a forest management practice known as green-timber reservoir management, or GTR. Trees in both sites are primarily a mix of various hardwoods, such as oak and hickory, and *Pinus* species, mainly loblolly pine, *Pinus taeda*, and longleaf pine, *Pinus palustris*. Wall Doxey Park is more of an upland mixed oak-hickory habitat, characterized by deep ravines and steep slopes. The underbrush in both sites comprises thorny *Smilax* and *Vaccinium* species, while the ground has thick leaf litter cover and fallen logs. Each site has small clearings with sparse canopy and clumps of *Panicum* and other grasses.

Although no quantitative or systematic host surveys were conducted, mammalian host species visually observed at both sites during the study included white-tailed deer, *Odocoileus virginianus*, and gray squirrels, *Sciurus carolinensis.* Reptilian hosts observed in abundance were five-lined skinks, *Plestiodon fasciatus*, broad-headed skinks, *Plestiodon laticeps*, and eastern fence lizards, *Scleroporus undulatus.* The most frequently encountered passerine bird hosts were house wrens, *Troglodytes aedon*, house sparrows, *Passer domesticus*, northern mockingbirds, *Mimus polyglottos*, and thrushes in the family Turdidae. Non-passerine bird species such as wild turkeys, *Meleagris gallopavo*, were also visually observed at both sites.

Tick collection and identification. During the one-year period from August 1, 2010 through July 31, 2011, ticks were collected by dragging a 1-m^2 corduroy cloth at the two sites. Both sites were visited once per 7-day period for one year, for a total of 104 site visits. Temperature, sky condition, and relative humidity were recorded on each collecting date, and collecting attempts were made each visit regardless of weather conditions. In order to collect from the plots in an efficient manner, each site was visually divided into 25 lanes and collecting was performed by transecting the sites in these predetermined lanes. The drag cloth was checked for ticks every 10 m, and all ticks found attached to the cloth were removed and placed in vials containing 95% ethanol for subsequent disease agent analysis (a separate study). Before each site visit, a random lane was chosen for slower, more intensive dragging. Vegetation and leaf litter were sampled more thoroughly in the selected lane by "scrubbing" the fabric into the litter, and the cloth was checked every five m. Due to low numbers of all life stages of *I. scapularis* and collecting discrepancies, it was not possible to statistically analyze these intensively sampled lanes for increased numbers of *I. scapularis*. However, as other

studies have noted, *I. scapularis* can be difficult to collect in the southern U.S.A. (Mackay & Foil 2005, Goddard & Piesman 2006), and based on the limited data obtained from this study, no conclusions can be drawn as to whether our intensive flagging efforts were any more effective in collecting *I. scapularis* at these sites.

In the laboratory, adult *I. scapularis* were counted, then identified to species and sex using the key of Keirans and Litwak (1989). All adult *I. scapularis* collected in April and May were checked to ensure that they were not *I. affinis* Neumann, a closely related species that is active in summer and whose range partially overlaps that of *I. scapularis.* All *Ixodes* nymphs collected were sent to Dr. Richard G. Robbins (Armed Forces Pest Management Board, Washington, DC) for identification to species. One *I. scapularis* nymph and one adult male and female pair have been deposited in the Mississippi Entomological Museum, Mississippi State University (voucher specimen numbers 80-2r and 80-2s).

Statistical analyses. The data analysis for this study was generated using SAS software, Version 9.2 of the SAS System for Windows (© 2008 SAS Institute Inc.). Collection data for adult ticks from both sites were analyzed to determine whether the number of *I. scapularis* adults collected was a function of temperature or vapor pressure deficit (VPD) (Diuk-Wasser *et al*. 2010). Collection data for nymphs were not analyzed due to low collection numbers (n = 3 at each site). Data from both sites were pooled for weeks 11–45, and since PROC UNIVARIATE indicated an unacceptable level of skewness (1.7173), data were transformed using log. This brought the skewness to 0.4630, which is within the acceptable range of (-1 to 1). A General Mixed Model Analysis using the PROC GLIMMIX procedure of SAS was then performed on log of (count +1) ticks. This model was chosen because it is more robust.

Results and discussion

Over the course of one year, 104 collection attempts were made and a total of 256 adult and 6 nymphal *I. scapularis* were collected from the two collection sites. From Wall Doxey State Park, 233 adults were collected, while only 23 were collected from Noxubee National Wildlife Refuge. The lower number of *I. scapularis* collected at Noxubee NWR may have been due to limited sampling at times because of heavy rainfall and flooding. The impact of removing ticks from both study sites during each collecting period is unknown. Three nymphal *I. scapularis* were collected from each collecting site, and no larval *I. scapularis* were collected at either site.

The first adult *I. scapularis* was collected during the 12^{th} week of collecting on October 19, 2010 at Wall Doxey, and the second adult *I. scapularis* was collected 3 days later on October 21, 2010 at Noxubee. The last adults were found on April 3, 2011 at Noxubee NWR during week 36 of collecting, and on May 25, 2011 at Wall Doxey during week 42 of collecting. The most adults were caught in March (97 adults, 37.9% of all *I. scapularis* collected), and no adults were collected from June through mid-October.

The first *I. scapularis* nymph was collected on August 2, 2010 at Wall Doxey Park. Two more nymphs were collected at Noxubee NWR on September 3, 2010 and September 16, 2010. The next nymph was collected on March 14, 2011 at Noxubee NWR, then two more on May 17, 2011 at Wall Doxey Park.

Results of the Analysis of Variance on these data are provided in Table 1. No significant correlation was found between VPD and number of ticks collected, or temperature and VPD combined and number of ticks collected. The equation used was: log (count +1) = -1.511 + 0.2175 week – 0.0039 $week^2$. $R^2 = 0.43$.

TABLE 1. Statistical analysis of adult *Ixodes scapularis* questing activity in relation to vapor pressure deficit (VPD). Asterisks depict interaction of two or more variables.

Source of Variation	Num DF	Den DF	F Value	Pr > F
Week	1	52	0.000	0.9480
VPD	1	52	1.87	0.1772
Week*VPD	1	52	0.39	0.5336
Week*Week	1	52	8.08	0.0064
Week*Week*VPD	1	52	2.86	0.0968

Results of this study are consistent with previous studies in which attempts to collect *I. scapularis* larvae and nymphs in the southern U.S. by dragging have yielded low numbers of adults and even lower numbers of larvae and nymphs (Goddard 1986, Piesman 2002, Mackay & Foil 2005, Diuk-Wasser *et al*. 2012). During 29 hours of dragging, Goddard and Piesman managed to collect only 12 *I. scapularis* larvae in mid-June in Mississippi (Goddard & Piesman 2006), but even those collection numbers are rare in the southern U.S.A. In the present study, other *Ixodes* nymphs were collected (18 *I. brunneus* Koch and 6 *I. dentatus* Marx), so we feel confident that the collecting method did not exclude nymphal *I. scapularis*. *Ixodes scapularis* is not a highly mobile tick, with hatching larvae actively dispersing only about 2–3 m from the egg mass (Stafford 1992), so collecting 256 adult *I. scapularis* between the two sites is indicative that a corresponding number of larvae and nymphs must also be present somewhere in the environment. Possible reasons for their absence during drag cloth sampling in the southern U.S.A. are: 1) collecting attempts made during times when they are not active, 2) immature *I. scapularis* are somehow not able to attach or remain on drag cloths, 3) other vegetation blocks or prevents contact with the cloth, 4) immature *I. scapularis* do not quest high enough in vegetation to make contact with the cloth, and 5) suitable lizard hosts may be so abundant that immatures attach to them rapidly and therefore do not need to quest very long.

Most likely, immature *I. scapularis* are difficult to collect with a drag cloth due to the fact that they spend most of their time deep in the leaf litter, using lizards as hosts (Piesman 2002). Rogers noted that *I. scapularis* larvae in Florida tend to stay close to the leaf litter rather than climbing up grass or foliage, as is common among other ixodid ticks (Rogers 1953). If immature *I. scapularis* are relying on lizards, which move freely through leaf litter, remaining in leaf litter would allow for more potential contact with these hosts. Considering the documented presence of *I. scapularis* adults in northern Mississippi and the non-corresponding low numbers of *I. scapularis* larvae and nymphs collected during this study, more research is warranted to clarify the times, vegetation types, and questing behavior of immature *I. scapularis* in the southern U.S.A.

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