Nymphs of the western black-legged tick (*Ixodes pacificus*) collected from tree trunks in woodland-grass habitat

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ABSTRACT: Nymphs of the western black-legged tick, *Ixodes pacificus*, were found on the trunks of trees during spring and summer in northwestern California. In a woodland-grass habitat, large- and medium-sized (>130 cm and 80 - 130 cm in circumference, respectively), moss-covered oak (*Quercus* spp.) trees supported ticks significantly more often than trees without these characteristics. Additionally, trees with basal leaf-litter and lacking shade (at time of sampling) were significantly associated with the presence of ticks. Mean tick-burdens were >1 for all oaks (1.06), all trees with basal leaf-litter (1.05), and all trees of large-circumference (1.19); 0.79 ticks per tree were collected over the entire study. Moss reduced the surface temperature of trees by a mean of 1.9°C (range of 1.6 - 5.0°C) and increased relative humidity by up to 2.5% from the ambient. These microclimatic changes, along with the presence of refugia in bark and western fence lizard (*Sceloporus occidentalis*) hosts on the lower-most surface of trees, likely accounted for ticks questing on the trunks. Although of undetermined epidemiological significance, the presence of host-seeking *I. pacificus* nymphs on tree trunks may shed light on the relation of abiotic and biotic factors to the life history of this important vector of disease.


Keyword index: Microhabitat, *Quercus* spp. oak, vector ecology.

INTRODUCTION

The microhabitats of ticks are determined by various intrinsic and extrinsic factors, including those that influence survival and enhance the likelihood of encountering a suitable host. In fact, micro-, meso-, and macrohabitats differentially provide environmental conditions to which ticks often are highly adapted and restricted (Milne 1944, Daniel and Dusbabek 1994, Sonenshine 1994, Duffield and Bull 1996). The geographic and biocenotic areas defined by these parameters, in turn, determine the focus of tick-transmitted microbial disease agents (Pavlovsky 1939, Falco and Fish 1988, Barker et al. 1992, Sonenshine 1993, Fish 1995).

In the northeastern United States, the distribution of the black-legged tick, *Ixodes scapularis* (formerly *I. dammini*), and concomitantly that of the Lyme disease spirochete, *Borrelia burgdorferi* sensu stricto, are determined by the presence of the tick’s hosts and specific microclimatic requirements provided in deciduous brush and woodland habitats (Wilson et al. 1985, Piesman and Gray 1994). This distribution has a vertical component inasmuch as *I. scapularis* ascends vegetation while host-seeking (Spielman et al. 1985). Further, Carroll (1996) flagged *I. scapularis* larvae from oak (*Quercus* spp.) trees and their basal leaf-litter and linked the occurrence of ticks on trees to semi-arboreal hosts and favorable microenvironments thereon. The western black-legged tick, *I. pacificus*, which is a vector of *B. burgdorferi* sensu lato, also has biocenotic relationships with woodland leaf-litter and tree-frequenting hosts in the far-western United States (Lane and Loye 1989, Tälleklint-Eisen and Lane 2000), but has not been associated with tree trunks previously. Therefore, we sampled tree trunks in a woodland-grass habitat for the presence of *I. pacificus* during its seasonal period of subadult activity in northwestern California.

MATERIALS AND METHODS

The study was conducted in a woodland-grass habitat in the foothills of the Coastal Range of Mendocino County at the Hopland Research and Extension Center (HREC). Located at elevations of between 700 and 800 m, the 2 study sites were comprised of moderately sloped hills interspersed with intermittent water...
sources, ravines, log-litter, and rocky areas. Woody vegetation was primarily composed of Quercus kelloggii, Q. wislizenii, and Q. agrifolia oak, Pacific madrone (Arbutus menziesii), and California bay (Umbellularia californica) trees amid annual grass and forb ground cover. The canopy cover was visually estimated at ca. 40% at both sites.

Although the activity of larval and nymphal I. pacificus peaks during spring to mid-summer (Lane and Loye 1989, Manweiler et al. 1992), vegetation at the study sites often remains wet and difficult to flag during early spring. Therefore, on 4 occasions in late spring and summer 1999 - 2000, trees were sampled for ticks in 2 areas located within 1 km of one another that were part of a study of avian and lizard tick-parasitism (Słowik and Lane 2001). A 1-m² flannel flag was brushed and wrapped around the lowest 1-m of each tree trunk, the area determined by Carroll (1996) to harbor the most I. scapularis ticks. Sixty trees at each of the 2 sites were flagged for ca. 20 s each during 1 sampling period in late-morning (AM) and late afternoon (PM), when the activity of questing ticks is high (Lane et al. 1985). Each tree was selected randomly by a roll of 2 dice that determined directional movement between trees on the ground; therefore, some trees were flagged more than once, but at least 2 wk apart. The type (genus or species), relative size (i.e., small, medium, or large trunk circumference of 50 - 80 cm, 80 - 130 cm, and >130 cm, respectively), and physical characteristics of each tree, such as the presence or absence of moss and shade (at time of sampling) on the trunk, and leaf-litter at the base, were recorded. During each sampling effort, 5 ambient and surface temperature recordings were taken at each of 10 trees. Additionally, relative humidity (RH) measurements were taken from the surfaces of 60 moss-covered and 60 non-moss-covered trees using a hygrometer (model HI 8564, Hanna Instruments, Woonsocket, Rhode Island) in August 2000, after it was determined that moss was associated with the presence of ticks and could influence the relative humidity of trunk microhabitats.

Flagged ticks were placed in small plastic vials containing a blade of grass for moisture, transported to the laboratory, and identified to species and stage according to the criteria of Furman and Loomis (1984). Because the prevalence of infection with B. burgdorferi in subadult I. pacificus at the HREC generally is low (Lane and Loye 1989, Tälleklint-Eisen and Lane 1999), ticks were not tested for spirochetal infection.

Multi-way Chi-square contingency tables (CT; 2-tailed) were used to determine any associations between the size of tree, type of tree, trunk moss, and basal litter. Two by two CT (2-tailed) were used to compare the relative numbers of oak vs. non-oak, moss- vs. non-moss-covered, basal litter vs. non-litter, shaded vs. unshaded, and AM- vs. PM-sampled trees yielding 1 or more ticks vs. those producing none. A 2 x 3 CT also was used to compare the numbers of small, medium, and large-sized trees with and without ticks. Due to differences in sample sizes in these categories of trees, mean numbers of ticks by type, moss, leaf-litter, shade, time of sampling, and size status were compared with non-parametric tests approximated to the normal curve (2-sample, Wilcoxon rank-sum test) or to the Chi-square distribution (3-sample, Kruskal-Wallis test). Tukey-type multiple comparisons then were used to detect differences in 3-sample comparisons. Data on RH were compared for trees that were shaded or unshaded during sampling, with and without trunk-moss, and sampled in AM and PM using Student’s or Welch’s approximate t-test (2-tailed) depending on the variance of the distributions. All analyses were performed with SAS software (1990) and following Zar (1999), with an alpha value of 0.05.

RESULTS

Tree Sampling

Approximately 71% (425/600) of the individual trees sampled were Quercus spp., whereas 25% (151) were madrone and 4% (24) were California bay (Table 1). Overall, slightly more than half (336/600) of the trees had a medium-sized circumference, nearly 70% (412) had moss on their trunks, two-thirds had basal leaf-litter (396), and 60% (358) were shaded at the time-of-flagging (Table 1). The presence of moss, basal litter, type, and size of tree were interdependent (all tests with X² > 28.0, P < 0.002). Sampled oak trees were most likely to be medium-sized, medium-sized trees most likely to be covered with moss, and moss was associated with basal leaf-litter in the highest proportions. Sampled madrones were least likely to have moss, whereas bay trees had litter, but were not moss-covered, in highest numbers.

Meteorological Data

Ambient AM and PM temperatures did not differ significantly, yet moss lowered temperatures at the tree-surface an average of 1.9°C (range, 1.6 - 5.0°C) from the ambient and shade decreased surface temperatures by an average of 2.1°C (range, 0 - 3.6°C; Table 1). In August 2000, moss increased RH at the tree surface by an average of 0.3% (range, 0.25%) over the ambient, an effect that was not detected on non-moss trees. Increases in the RH of moss-covered trunks did not
Table 1. Descriptive data on trees sampled for *I. pacificus* ticks in late spring and early summer in southeastern Mendocino County, California, 1999 and 2000 (120 trees flagged each period).

<table>
<thead>
<tr>
<th>Sampling period</th>
<th>Average temperature*</th>
<th>% tree-typea</th>
<th>% trees of particular sizeb</th>
<th>% moss-covered</th>
<th>% with basal leaf-litter</th>
<th>% shaded</th>
<th>Mean no. ticks/tree</th>
</tr>
</thead>
<tbody>
<tr>
<td>mid-June 1999</td>
<td>30.5</td>
<td>71.7K, 28.3M</td>
<td>29.2; 63.4; 7.4</td>
<td>70.8</td>
<td>67.5</td>
<td>57.2</td>
<td>0.86</td>
</tr>
<tr>
<td>late June 1999</td>
<td>33.8</td>
<td>29.2K, 63.4M, 7.4B</td>
<td>19.2; 45.8; 35.0</td>
<td>62.5</td>
<td>47.5</td>
<td>56.7</td>
<td>0.08</td>
</tr>
<tr>
<td>early July 1999</td>
<td>32.3</td>
<td>61.7K, 23.3M, 15.0B</td>
<td>15.9; 57.4; 26.7</td>
<td>55.8</td>
<td>63.3</td>
<td>56.7</td>
<td>0.10</td>
</tr>
<tr>
<td>late May 2000</td>
<td>29.0</td>
<td>79.2K, 19.2M, 1.6B</td>
<td>24.2; 60.0; 15.8</td>
<td>78.4</td>
<td>75.9</td>
<td>62.5</td>
<td>1.66</td>
</tr>
<tr>
<td>early June 2000</td>
<td>24.5</td>
<td>76.7K, 23.3M</td>
<td>24.2; 53.3; 22.5</td>
<td>75.9</td>
<td>75.8</td>
<td>65.9</td>
<td>1.24</td>
</tr>
</tbody>
</table>

*Average of ambient AM and PM temperature in °C; a K = *Quercus* spp. oak, M = madrone (*Arbutus menziesii*), B = bay (*Umbellularia californica*); b large tree-circumference of >130 cm; medium circumference of 80-130 cm; small circumference of 50-80 cm.

Table 2. Mean numbers of *Ixodes pacificus* nymphs collected per tree in spring and summer in woodland habitat in southeastern Mendocino County, California, 1999 and 2000.

<table>
<thead>
<tr>
<th>Sampling period*</th>
<th>Oak/non-oakc</th>
<th>With moss/without mossb</th>
<th>With basal litter/without litter</th>
<th>Shaded/unshadedc</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>mid-June 1999</td>
<td>1.04/0.16</td>
<td>1.09/0.29</td>
<td>1.10/0.36</td>
<td>1.01/0.65</td>
<td>0.86</td>
</tr>
<tr>
<td>late June 1999</td>
<td>0.120</td>
<td>0.120</td>
<td>0.16/0</td>
<td>0.01/0.17</td>
<td>0.08</td>
</tr>
<tr>
<td>early July 1999</td>
<td>0.15/0.02</td>
<td>0.16/0.02</td>
<td>0.16/0</td>
<td>0.06/0.21</td>
<td>0.10</td>
</tr>
<tr>
<td>late May 2000</td>
<td>1.95/0.56</td>
<td>1.84/1.00</td>
<td>2.03/0.48</td>
<td>1.36/2.16</td>
<td>1.66</td>
</tr>
<tr>
<td>early June 2000</td>
<td>1.40/0.72</td>
<td>1.41/0.72</td>
<td>1.45/1.15</td>
<td>0.79/0.79</td>
<td>1.24</td>
</tr>
</tbody>
</table>

*a 120 trees sampled each period; b Madrone (*Arbutus menziesii*) and bay (*Umbellularia californica*) trees; c Tree without moss on lower trunk; Trunk unshaded at time of sampling.
differ significantly when compared by time-of-day: the mean increase in AM was 0.3% and that of PM 0.2% above the ambient. Shade did not consistently impact RH, as measurements taken near and on the surfaces of trees varied haphazardly.

**Tick Sampling**

In total, 471 ticks were collected from 29.8% (179/600) of the tree trunks, which represented an overall mean of 0.79 ticks per tree (Table 2). Over 99% (469) were *I. pacificus* nymphs; 2 *I. pacificus* adults also were collected. The different categories of trees accounted for the following percentages of ticks: oak, 91.5% (431) and non-oak, 8.5%; moss, 87.9% (414) and non-moss, 12.1%; litter, 89.4% (421) and non-litter, 10.6%; shaded, 49% (231) and unshaded, 51%. Trees of medium-circumference harbored ca. 54% (254) of all ticks, whereas large and small trees supported 35.2% (166) and 10.8% (51) of the ticks, respectively.

Significantly more oak than non-oak ($X^2 = 43.2, df = 1, P < 0.001$), moss-covered than non-moss covered ($X^2 = 29.5, df = 1, P = 0.002$), litter than non-litter contacting ($X^2 = 49.2, df = 1, P < 0.001$), and unshaded than shaded ($X^2 = 5.1, df = 1, P < 0.025$) trees harbored at least 1 tick on their trunks. AM- and PM-sampled trees with and without ticks were similar in number. The mean tick-burdens were significantly larger for oak trees with and without ticks were similar in number.

**DISCUSSION**

In northwestern California, host-seeking *I. pacificus* nymphs use woodland trees as a questing platform in apparent similarity to ixodids in the eastern United States (Carroll 1996). In Maryland, *I. scapularis* larvae were more likely to be found in highest numbers on oak trees and those having large diameters in a mixed deciduous forest (Carroll 1996). Furthermore, certain species and sizes of trees were more likely to provide favorable habitat for ticks or their hosts in the form of bark, moss, and crevices; however, these physical characteristics were associated on sampled trees. In the present study, large- and medium-circumference oaks had deeply fissured bark, more and thicker moss, and basal litter when compared to other trees, and likewise supported higher tick numbers. In contrast, madrone and bay trees usually had no moss (142/175) and typically possessed relatively smooth bark. Although *I. pacificus* appears to be associated with microhabitats provided by individual trees rather than with specific genera or species of trees, the potential attractiveness or repellency of each tree’s phytochemicals for ticks remains undefined (Carroll 1996). Indeed, associations between a European vector of disease, *I. ricinus*, and certain genera of trees have been found in lowland forests (Rosicky and Hejny 1961), as have been determined for *Amblyomma americanum* in Oklahoma woodlands (Semtner et al. 1971).

Trunk-moss, and probably to a lesser extent fissured bark, reduces temperatures and increases microhumidity levels, factors that critically influence tick activity and survival (Lees 1969, Sonenshine 1993, Daniel and Dusbabek 1994). Diel activity of *I. pacificus* nymphs in northwestern California is positively correlated with RH and negatively correlated with soil temperature, and these ticks quest in shaded environments (Lane et al. 1995). Shade appeared to have had an equivocal effect on the activity of *I. pacificus* nymphs in the current study, however, which may be due to its diurnally changing presence on trunks. On the other hand, moss-covered trunks seemed to support all the microclimatic needs of host-seeking nymphs. In fact, the use of moist, humid microenvironments within vegetational strata during questing is common to exophilic ixodids. Dermacentor
variabilis immatures, for example, use the roting leaf-layer of the forest floor as a substrate from which to find hosts (Sonenshine 1991).

Although the average increase in RH in the moss layer was small, it may have been mitigated by the relatively high temperatures and low ambient RH in exposed woodland areas in late summer. This increase is likely to be more significant during winter and early spring when precipitation and ambient RH are high and trunk-moss is more moist. However, mosses appear to vary widely in their ability to affect ambient RH: some mosses did not alter RH, whereas others increased RH by as much as 2.5%. In future studies, alternative measures of water-content such as vapor pressure might better indicate the true levels of moisture provided by trunk moss.

It remains to be determined whether the moss-induced changes in RH of the magnitude noted in our study influence the longevity and activity of nymphal I. pacificus on woodland trees at the HREC. Ticks differ greatly, by both species and life-stage, in their ability to cope with the loss of water to the environment, but immatures are particularly vulnerable to dessication via decreased RH (e.g., Knülle and Rudolph 1982, Sonenshine 1991, Peavey and Lane 1996). Stafford (1994) found that almost all larval I. scapularis held at RH of 65% and 75% in the laboratory died within 48 hr, whereas nymphs survived only 8 d under these conditions. The presence of questing I. pacificus nymphs in moss, especially their apparently exclusive occupation of trees with moss during the climatic inhospitability of late summer, indicates that these microenvironments promote the survival of replete, molting larvae. In fact, the larval molt accounts for the highest mortality in the life-cycle of ticks and larval dispersion after detachment from its host is extremely limited (Loye and Lane 1988, Sonenshine 1994). Additionally, the presence of questing nymphs in late summer, when harsh environmental conditions prevent tick activity on the exposed ground and tick burdens on hosts are extremely low (Manweiler et al. 1992, Lane et al. 1995), implies that the host-seeking period may be prolonged for certain I. pacificus associated with tree trunks.

Our results also indicate that nymphs may be ascending the lower portion of the tree trunk from basal litter, as suggested for I. scapularis larvae by Carroll (1996), or using moss as an extension of the ground surface or its vegetational layer to quest for hosts. Additionally, nymphs may be transported onto trunks by the western fence lizard, Sceloporus occidentalis, which frequents trees and logs, abounds in woodland-grass, and is a primary host to immature ticks (Lane and Loye 1989, Block and Morrison 1998) or Peromyscus spp. mice, which are also arboreal (e.g., Nicholson 1941). Chemicals from these hosts that remain on the tree trunks also may influence tick activity (Carroll 1996).

Late spring/early summer maxima and mid-summer minima in tick numbers on trees also approximate the period of highest questing activity of nymphs in leaf-litter and lizard-burdens in this region (Lane and Loye 1989, Manweiler et al. 1992, Clover and Lane 1995). The absence of larvae and small number of adults flagged in our study appear to support previous research that indicates a slightly earlier seasonal peak in larval vs. nymphal activity, i.e., April and May (Lane and Loye 1989, Manweiler et al. 1992), and adult host-seeking that occurs primarily in winter (Lane 1990). Likewise, Carroll (1996) collected only I. scapularis larvae from tree trunks in late summer when only this stage is active (Yuval and Spielman 1990).

The significance of tree trunks in the ecology of I. pacificus and, perhaps the enzootiology of B. burgdorferi s.l., awaits clarification. However, trunks with certain characteristics appear to offer I. pacificus nymphs refugia and questing opportunities that may rival those found at ground level. The association of I. pacificus with trees is probably important to understanding the tick’s life-cycle in relation to abiotic (e.g., temperature and humidity requirements) and biotic (e.g., host distribution and behavior) factors. However, it appears less critical to understanding the transmission-risk of tick-borne disease due to the infrequent human contact with trees, inability of ticks to jump or fall on people from trees, and the presence of preferred, reservoir-incompetent hosts such as the western fence lizard on the tree trunks.

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