OXFORD

Short Communication

The Relationship Between Soil Arthropods and the Overwinter Survival of *Ixodes scapularis* (Acari: Ixodidae) Under Manipulated Snow Cover

J. C. Burtis,[1,2] R. S. Ostfeld,[3] J. B. Yavitt,[1] and T. J. Fahey[1]

[1]Cornell University, Department of Natural Resources, Fernow Hall, Ithaca, NY 14853 (jb766@cornell.edu; jby1@cornell.edu; tjf5@cornell.edu), [2]Corresponding author, e-mail: jb766@cornell.edu, and [3]Cary Institute of Ecosystem Studies, 2801 Sharon Turnpike, Millbrook, NY 12545 (ostfeldr@caryinstitute.org)

Abstract

We explored the relationship between the diversity and abundance of the soil arthropod predator community and the overwinter survival of engorged larval *Ixodes scapularis* Say under variable snow cover in a hardwood forest. We reduced the snow cover over 30 soil core field microcosms, simulating predicted changes in snow pack in the northeastern United States. An additional 29 microcosms were used as references with no snow pack manipulation. Each microcosm contained 15 engorged larval *I. scapularis*. We expected lower soil temperature without insulating snow cover to reduce tick survival. However, we observed that reduced snow cover had no effect, with 44.2 and 44.7% overwintering successfully in the reference and snow-removal plots, respectively. Increasing taxonomic family richness of arthropod predators and the total number of large (>1 mm) arthropod predators significantly reduced the overwinter survivorship of *I. scapularis* within the microcosms. Small (<1 mm) arthropod predator abundance had no effect. Our results suggest that forests with complex natural arthropod predator communities show reduced tick survival.

Ixodes scapularis (blacklegged tick) Say is the primary vector for many widespread zoonotic diseases in the United States, including Lyme disease, anaplasmosis, and babesiosis. This important disease vector spends >95% of its 2-yr life cycle on or in the soil, but the impact of the soil environment on its survival is understudied, particularly over the winter. Most research regarding *I. scapularis* has focused on interactions with hosts (Ostfeld et al. 2001, Keesing et al. 2009, Kilpatrick et al. 2014), or during their summer and fall activity peaks (Stafford 1994, Berger et al. 2014). With few exceptions (Lindsay et al. 1995, Brunner et al. 2012), factors impacting their overwinter survival in the field have been neglected (Ostfeld and Brunner 2015). Additionally, arthropod predators are known to eat *I. scapularis* in laboratory settings (Samish and Alexeev 2001, Samish et al. 2004), but the impact of arthropod predators, many of which are widespread generalists (Scheu 2001, Digel et al. 2014), has received little investigation.

We explored the impact of snow cover and the composition of the soil arthropod community on the overwinter survival of engorged larval *I. scapularis* as they molted into nymphs in the field. Larvae emerge and feed in late summer, molt into nymphs in the autumn, and then emerge to feed in the late spring the following year (Ostfeld et al. 1996). Laboratory trials have shown that engorged larval *I. scapularis* survival is greatly reduced by temperatures below −10°C (Burks et al. 1996, Vandyk et al. 1996), but the time scale of these trials was hours rather than weeks or months. Moreover, laboratory conditions often prevent ticks from behavioral adaptations that are available in the field. Snow cover can insulate the soil, and may protect ticks from cold as it does with many other soil-dwelling arthropods (Templer et al. 2012). Snow pack is predicted to decrease in the northeastern United States, with reduced early winter snowfall (Hayhoe et al. 2008, Campbell et al. 2010), possibly decreasing soil temperatures throughout the winter (Hardy et al. 2001).

Snow cover removal affects soil arthropod communities (Templer et al. 2012), and recent research suggests that changes in soil food web structure can cascade to influence blacklegged tick populations (Coyle et al. 2013, Burtis et al. 2014). Interactions between snow cover manipulation and soil arthropod communities may affect the survivorship of blacklegged ticks. We tested the impact of reduced snow cover, simulating predicted conditions in the northeastern United States, on the overwinter survival of *I. scapularis*, and examined the role of arthropod predator richness and abundance on overwinter survival under reduced snow cover.
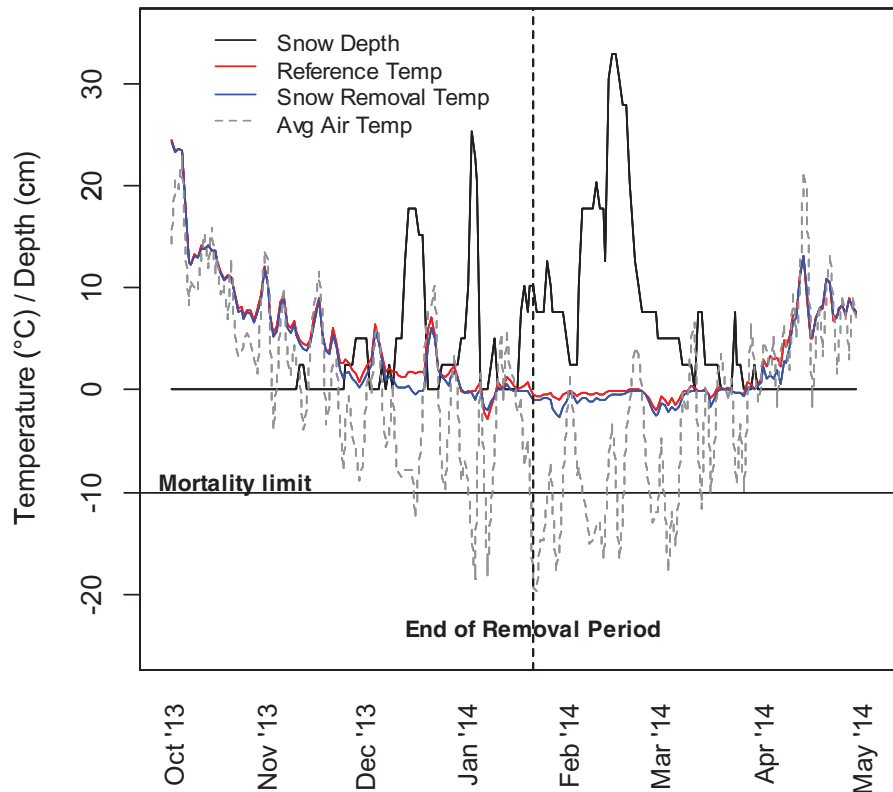
Fig. 1. Mean daily temperatures recorded at the litter–soil interface from the data loggers in the snow removal (blue) and reference (red) plots, along with the average air temperature (dashed-grey), and snow depth on the reference plot (black). The vertical dashed line signifies the end of the snow-removal period. The horizontal solid line represents the temperature at which *I. scapularis* mortality begins to increase substantially (Vandyk et al. 1996). The difference in mean snow depth between the plots after February 4th was 0.12 cm.

Materials and Methods

Collecting and Rearing of *I. scapularis*

Larval *I. scapularis* were collected at the Cary Institute of Ecosystem Studies in Millbrook, NY (41° 47′5.13″ N; 73° 44′0.83″ W) in July and August (2013) using a dragging method. Larvae were fed on 18 wild-type *Peromyscus leucopus* from the University of South Carolina's *Peromyscus* Genetic Stock Center. The engorged larvae were placed in humidified vials, and stored at 5°C until they were placed in the field in September 2013. Engorged larvae from individual mice were mixed randomly before placement into microcosms.

Field Installation

Our field installation was located in a northern hardwood forest near Ithaca, NY (42° 28′4.06″ N; 76° 25′34.21″ W). The 20- by 10-m installation contained 63 soil core microcosms. Each microcosm was wrapped in fine-mesh cloth and contained within a PVC pipe (10 cm diameter by 5 cm deep) which enclosed soil and leaf material from the site, as well as the associated arthropod community. Additional information regarding microcosm construction is available in Brunner et al. 2012. Microcosms were clustered into a snow-removal group (30 cores), and a reference group (29 cores) where snow cover was not manipulated. Two TidbiT HOBO temperature data loggers, in larvae-free microcosms, were placed in each group (four total). Data loggers were positioned directly under the leaf litter and recorded temperatures every 4 h. All nondata logger microcosms contained 15 engorged *I. scapularis* larvae.

Snow was removed from half the plot between 12 November 2013 and 21 January 2014 to simulate predicted snowfall pattern changes in the northeastern United States (Hayhoe et al. 2008, Campbell et al. 2010). All snow was removed except for the first centimeter, which was packed down to minimize leaf litter disturbance. Snow depth was recorded twice a week at 8 points on each plot. Air temperature was recorded at the Northeastern Regional Climate Center, located 4.5 km from our Ithaca field site.

Collection of *I. scapularis* and Other Arthropods

I. scapularis and other arthropods were collected from the microcosms between 3 July and 28 July 2014. Soil and leaf materials from each microcosm were hand sorted for 2 h (Supp Fig. 1 [online only]). Afterward, all materials were placed in Berlese funnels for 7 d to collect additional nymphs and arthropods. All arthropods were sorted to taxonomic order. Arthropod predators in the class Chilopoda and the orders Araneae, Mesostigmata, Pseudoscorpionida, and Trombidiformes were sorted to taxonomic family (Dindal 1990, Ubick and Dupérré 2005, Krantz and Walter 2009).

Statistical Analyses

We used two polynomial models to examine differences in daily mean soil temperatures between the snow removal and reference plots: 1) examined temperature differences during the snow-removal period, and 2) differences outside the snow-removal period. Both models included "snow removal" as a factor, and "date" as a third-order polynomial. We explored the impact of snow removal on *I. scapularis* survival with a Student's *t*-test.

We ran three mixed-effects linear models examining the relationship between the litter community and *I. scapularis* overwinter survival including the effects of predator family diversity, large
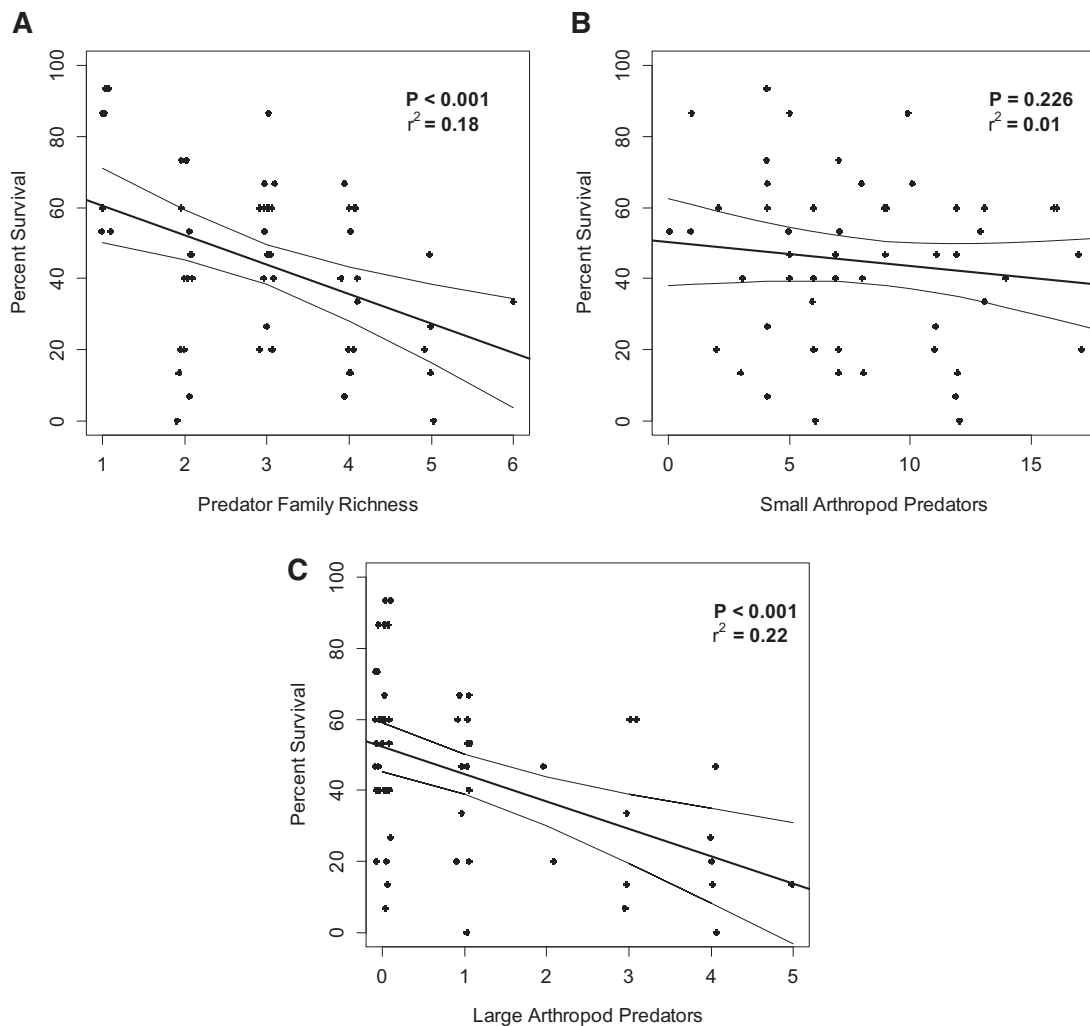
Fig. 2. The percentage of engorged larval *I. scapularis* that overwintered successfully plotted against—(A) arthropod predator family richness, (B) small arthropod predator abundance, and (C) large arthropod predator abundance, with best fit lines and 95% confidence intervals. Noise has been added to the data to make overlapping values visible.

(>1 mm) arthropod predator (Araneae or Chilopoda) abundance, and small (<1 mm) arthropod predator (Mesostigmata or Pseudoscorpionida or Trombidiformes) abundance. A fourth model examined the effect of predator family richness on large arthropod predator abundance. Collection date was included as a random effect, accounting for temporal variation in arthropod predator populations. *P*-values were corrected for multiple comparisons using a false-discovery-rate method (Benjamini and Hochberg 1995). Analyses were run in the statistical program "R" (R Core Team 2014).

Results

During the snow-removal period, snow depth averaged 4.33 cm (± 0.38) on the reference plot. Mean daily soil temperatures were 0.62°C lower in the snow removal compared with the reference plot during the snow-removal period ($F = 5.13$; df = 1, 137; $P = 0.025$), and there was no significant difference outside the snow-removal period ($F = 1.906$; df = 1, 271; $P = 0.169$). The lowest air temperature recorded was −19.75°C, while the lowest soil temperature was −4.23°C (Fig. 1). *I. scapularis* survival (percentage of nymphs recovered) was not impacted by snow removal ($t = 0.071$; df = 53;

$P = 0.944$), with 44.2% (± 4.96) and 44.7% (± 3.91) surviving in the snow removal and reference plots, respectively.

Arthropods from 17 taxonomic orders were found in our microcosms, and 13 arthropod predator families were identified (Supp Tables 1 and 2 [online only]). The dominant large arthropod predators were in the family Lithobiidae (Chilopoda). The dominant small arthropod predator family was Parasitidae (Mesostigmata). Arthropod predator family richness ($t = -3.59$, df = 57, $P < 0.001$; Fig. 2A) and large arthropod predator abundance ($t = -3.82$, df = 57, $P < 0.001$; Fig. 2C) both had a significant negative effect on *I. scapularis* survival. There was no relationship between small arthropod predator abundance and *I. scapularis* survival ($t = -1.23$, df = 57, $P = 0.226$; Fig. 2B). There was also a positive relationship between predator family richness and large arthropod predator abundance ($t = 5.42$, df = 57, $P < 0.001$; Table 1). Snow-removal treatment had no effect, and was not included in these analyses (ΔAIC<2).

Discussion

Snow removal had no impact on the overwinter survival of *I. scapularis* in our microcosms. Despite air temperatures below −19°C,

Table 1. Results of three linear mixed-effects models of factors affecting overwinter *I. scapularis* survival including—(A) arthropod predator family richness, (B) large (>1 mm) arthropod predator abundance, and (C) small (<1 mm) arthropod predator abundance

Factor	Estimate	SE	*t*-value	df	*P*-value
Model A					
Family richness	10.33	0.35	−3.59	1, 57	<0.001*
Model B					
Large predators	7.83	0.52	−3.82	1, 57	<0.001*
Model C					
Small predators	−0.11	0.09	−1.23	1, 57	0.2257
Model D					
Family richness	0.66	0.12	5.42	1, 57	<0.001*

Model D shows the effect of arthropod predator family richness on large arthropod predator abundance. Collection date was included in each model as a random effect. All *P*-values were corrected using Benjamini & Hochberg's false-discovery-rate method (Benjamini and Hochberg 1995).

temperatures under the leaf litter, where *I. scapularis* overwinters (Yuval and Spielman 1990, Daniels et al. 1996), never dropped below −4.23°C. Laboratory trials suggest survival of engorged larval *I. scapularis* is not strongly reduced until temperatures reach −10.83°C (Vandyk et al. 1996). Our snow-removal treatment had minimal impact on the soil temperature, partly because the snow cover during the removal period was intermittent (Fig. 1). It seems unlikely that predicted changes to winter soil temperature as a result of reduced snow cover in regions with intermittent early winter snow cover will have a strong direct effect on the overwinter survival of *I. scapularis*. Our results agree with previous field observations of the effect of temperature on *I. scapularis* overwinter survival (Lindsay et al. 1995, Brunner et al. 2012). It is possible that prolonged exposure to cold in areas with a permanent early winter snow pack, where snow removal has a stronger effect on soil temperature (Decker et al. 2003, Templer et al. 2012), would impact *I. scapularis* overwinter survival. More study is needed in these regions.

In contrast, soil arthropod predator community richness had a significant negative effect on nymphal survival (Fig. 2A), unrelated to snow-removal treatment. The density of large arthropod predators, predominantly centipedes in the family Lithobiidae, had a negative relationship with *I. scapularis* survival (Fig. 2C), while no such trend existed for small arthropod predators (Fig. 2B). It is possible that large arthropod predators targeted *I. scapularis* in our microcosms, while predatory mites were too small to attack *I. scapularis*. Additionally, we observed that as the arthropod predator communities grew more complex, as measured by family richness, more large arthropod predators were present. This may indicate that as soil communities grow more complex, they can support more large arthropod predators, as previously hypothesized (Chen and Wise 1999, Kalinkat et al. 2013).

The density and richness of the soil arthropod communities in our microcosms were lower than those observed in other studies (Burtis et al. 2014). The impact of some larger arthropods, particularly spiders, may actually be greater than that observed in our microcosms. Additionally, our lack of knowledge regarding interactions between *I. scapularis* and arthropod predatory taxa makes it difficult to directly link the decline in *I. scapularis* survival to the predators in the cores. Our data represent a snapshot of the arthropod community, as we could not detect inactive or dead arthropods. Thus, predators not active in July were unaccounted for, again leading to an underestimation of their potential impact.

The impact of the soil food web on *I. scapularis* survival has received little study, and our data suggest that it warrants further evaluation. It is increasingly evident that *I. scapularis* density can be impacted by many factors within the soil ecosystem (Coyle et al. 2013, Burtis et al. 2014). This study focused on one portion of the *I. scapularis* life cycle and is limited in scale, but it indicates that soil-dwelling arthropod predators may affect *I. scapularis* survival. Further studies may help clarify which factors affect the localized spatial distribution of this important disease vector.

Acknowledgments

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

References Cited

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B. 57: 289–300.

Berger, K. A., H. S. Ginsberg, L. Gonzalez, and T. N. Mather. 2014. Relative humidity and activity patterns of *Ixodes scapularis* (Acari: Ixodidae). J. Med. Entomol. 51: 769–776.

Brunner, J. L., M. Killilea, and R. S. Ostfeld. 2012. Overwintering survival of nymphal *Ixodes scapularis* (Acari: Ixodidae) under natural conditions. J. Med. Entomol. 49: 981–987.

Burks, C. S., R. L. Stewart, G. R. Needham, and R. E. Lee. 1996. The role of direct chilling injury and inoculative freezing in cold tolerance of *Amblyomma americanum*, *Dermacentor variabilis*, and *Ixodes scapularis*. Physiol. Entomol. 21: 44–50.

Burtis, J. C., T. J. Fahey, and J. B. Yavitt. 2014. Impact of invasive earthworms on *Ixodes scapularis* and other litter-dwelling arthropods in hardwood forests, central New York state, USA. Appl. Soil Ecol. 84: 148–157.

Campbell, J. L., S. V. Ollinger, G. N. Flerchinger, H. Wicklein, K. Hayhoe, and A. S. Bailey. 2010. Past and projected future changes in snowpack and soil frost at the Hubbard Brook Experimental Forest, New Hampshire, USA. Hydrol. Process 24: 2465–2480.

Chen, B., and D. H. Wise. 1999. Bottom-up limitation of predaceous arthropods in a detritus-based terrestrial food web. Ecology 80: 761–772.

Coyle, D. R., M. W. Murphy, S. M. Paskewitz, J. L. Orrock, X. Lee, R. J. Murphy, M. A. McGeehin, and K. F. Raffa. 2013. Belowground herbivory in red pine stands initiates a cascade that increases abundance of Lyme disease vectors. For. Ecol. Manag. 302: 354–362.

Daniels, T. J., R. C. Falco, K. L. Curran, and D. Fish. 1996. Timing of *Ixodes scapularis* (Acari: Ixodidae) oviposition and larval activity in southern New York. J. Med. Entomol. 33: 140–147.

Decker, K.L.M., D. Wang, C. Waite, and T. Scherbatskoy. 2003. Snow removal and ambient air temperature effects on forest soil temperatures in northern Vermont. Soil Sci. Soc. Am. J. 67: 1234–1242.

Digel, C., A. Curtsdotter, J. Riede, B. Klarner, and U. Brose. 2014. Unravelling the complex structure of forest soil food webs: higher omnivory and more trophic levels. Oikos 123: 1157–1172.

Dindal, D. L. 1990. Soil biology guide. John Wiley & Sons, New York, NY.

Hardy, J. P., P. M. Groffman, R. D. Fitzhugh, K. S. Henry, A. T. Welman, J. D. Demers, T. J. Fahey, C. T. Driscoll, G. L. Tierney, and S. Nolan. 2001.

Snow depth manipulation and its influence on soil frost and water dynamics in a northern hardwood forest. Biogeochemistry 56: 151–174.

Hayhoe, K., C. Wake, B. Anderson, X. Z. Liang, E. Maurer, J. Zhu, J. Bradbury, A. DeGaetano, A. M. Stoner, and D. Wuebbles. 2008. Regional climate change projections for the Northeast USA. Mitig. Adapt. Strategies Glob. Chang. 13: 425–436.

Kalinkat, G., U. Brose, and B. C. Rall. 2013. Habitat structure alters top-down control in litter communities. Oecologia 172: 877–887.

Keesing, F., J. Brunner, S. Duerr, M. Killilea, K. LoGiudice, K. Schmidt, H. Vuong, and R. S. Ostfeld. 2009. Hosts as ecological traps for the vector of Lyme disease. Proc. R. Soc. Lond. [Biol]. 276: 3911–3919.

Kilpatrick, H. J., A. M. Labonte, and K. C. Stafford. 2014. The relationship between deer density, tick abundance, and human cases of Lyme disease in a residential community. J. Med. Entomol. 51: 777–784.

Krantz, G. W., and D. E. Walter. 2009. A manual of acarology. 3rd ed. Texas Tech University Press, TX.

Lindsay, L. R., I. K. Barker, G. A. Surgeoner, S. A. McEwen, T. J. Gillespie, and J. T. Robinson. 1995. Survival and development of *Ixodes scapularis* (Acari: Ixodidae) under various climatic conditions in Ontario, Canada. J. Med. Entomol. 32: 143–152.

Ostfeld, R. S., and J. L. Brunner. 2015. Climate change and *Ixodes* tick-borne diseases of humans. Phil. Trans. R. Soc. B. 370: 2014–0051.

Ostfeld, R. S., K. R. Hazler, and O. M. Cepeda. 1996. Temporal and spatial dynamics of *Ixodes scapularis* (Acari: Ixodidae) in a rural landscape. J. Med. Entomol. 33: 90–95.

Ostfeld, R. S., E. M. Schauber, C. D. Canham, F. Keesing, C. G. Jones, and J. O. Wolff. 2001. Effects of acorn production and mouse abundance on abundance and *Borrelia burgdorferi* infection prevalence of nymphal *Ixodes scapularis* ticks. Vector Borne Zoonotic Dis. 1: 55–63.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Samish, M., and E. Alekseev. 2001. Arthropods as predators of ticks (Ixodoidea). J. Med. Entomol. 38: 1–11.

Samish, M., H. Ginsberg, and I. Glazer. 2004. Biological control of ticks. Parasitology 129: S389–S403.

Scheu, S. 2001. Plants and generalist predators as links between the below-ground and above-ground system. Basic Appl. Ecol. 2: 3–13.

Stafford, K. C. 1994. Survival of immature *Ixodes scapularis* (Acari: Ixodidae) at different relative humidities. J. Med. Entomol. 31: 310–314.

Templer, P. H., A. F. Schiller, N. W. Fuller, A. M. Socci, J. L. Campbell, J. E. Drake, and T. H. Kunz. 2012. Impact of a reduced winter snowpack on litter arthropod abundance and diversity in a northern hardwood forest ecosystem. Biol. Fert. Soils. 48: 413–424.

Ubick, D., and N. Dupérré. 2005. Spiders of North America: An identification manual. American Arachnological Society, MO.

Vandyk, J. K., D. M. Bartholomew, W. A. Rowley, and K. B. Platt. 1996. Survival of *Ixodes scapularis* (Acari: Ixodidae) exposed to cold. J. Med. Entomol. 33: 6–10.

Yuval, B., and A. Spielman. 1990. Duration and regulation of the developmental cycle of *Ixodes dammini* (Acari: Ixodidae). J. Med. Entomol. 27: 196–201.