EFFECTS OF HOSTS AND LEAF LITTER ON SEASONAL DECLINES IN BLACKLEGGED TICKS

MELISSA COLON
SUNY College at Old Westbury, Old Westbury, NY 11568 USA

MENTOR SCIENTISTS: DRS. RICHARD S. OSTFELD[1] AND FELICIA KEESING[2]
[1]*Institute of Ecosystem Studies, Millbrook, NY 12545 USA*
[2]*Bard College, Annandale-on-Hudson, NY 12504 USA*

Abstract. Some of the infectious diseases of wildlife are also transmissible to humans. One prime example of such a "zoonotic disease" is Lyme disease, the most common vector-borne disease in the United States. In recent years, Lyme disease has undergone dramatic increases in incidence (Ostfeld, 1997). This epidemic is mainly associated with seasonal peaks in host-seeking activity by ticks. Seasonal activity peaks are short-lived and followed by declines caused by two main factors: death of ticks that fail to find a host, or successful encounters with a suitable host. These two causes of seasonal declines have opposing effects on future tick numbers and hence Lyme disease risk. Using a factorial field experiment at the Institute of Ecosystem Studies, I investigated the causes of declines in host-seeking activity by larval and nymphal ticks, to distinguish the importance of these two factors. To estimate the fraction of seasonal attrition accounted for by finding a suitable host, host access to small plots was manipulated using fencing. It was also determined whether the presence of leaf litter would significantly decrease losses due to mortality, as leaf litter provides the humidity, temperature regulation and source of hosts required for tick survival. No significant differences in attrition of either larval or nymphal ticks in plots with leaf litter intact versus leaf litter removed, was observed. This observation weakens prior conclusions regarding the protective value of leaf litter, at least in closed canopy forests. For nymphs, but not for larvae, the exclusion of hosts decreased rates of attrition. The interactions between host availability and leaf litter treatments were not significant for either life stage. High host availability in the current year is likely to reduce current numbers of host-seeking ticks, and consequently risk of human exposure to tick-bites. However, because ticks require hosts to survive and become infected, high host availability is expected to increase subsequent tick abundance and Lyme-disease risk.

INTRODUCTION

Ticks are parasites that feed on the blood of vertebrate hosts by embedding their mouthparts into the skin of their hosts. This mechanism makes ticks ideal vectors or organisms for storing and transmitting tick-borne diseases, such as Lyme disease (Booth, 1999). Lyme disease is transmitted to humans by blacklegged ticks, *Ixodes scapularis*, infected with the spirochete bacterium, *Borrelia burgdorferi* (Ginsberg, 1994). In order to contract Lyme disease a tick must attach and feed on a human host for a minimum of 24 hours.

To adequately understand the infection process and the risk associated with it, it is vital to grasp the life cycle of the tick. There exist three active developmental stages: larva, nymph, and adult, each requiring a single blood meal before molting into its subsequent stage (Ostfeld, 1997). The infectious agent responsible for Lyme disease is typically acquired at the larval and nymphal stages and is transmissible to humans at the nymphal and adult stages. Larvae predominately hatch mid to late summer, uninfected, as transmission from mother to offspring is rare; and can contract *Borrelia burgdorferi* when they feed on an infected host. Nymphs, which are most active from late May to early July, are responsible for the majority of human cases, on account of their small size and activity peak which coincides with that of humans (Ostfeld et al. 2006). Larval and nymphal ticks prefer to feed off of the white-footed mouse, and with an infection rate of 40-90%, these two life stages have a higher probability of becoming infected (LoGuidine et. al. 2003). Adult ticks peak during the fall when human outdoor activity tends to dwindle and are far larger than nymphs, making them more visible and responsible for fewer cases of Lyme disease. Focus has been weighed toward larval and nymphal ticks due to the impact these two life

stages have on Lyme disease risk. Larval ticks have an indirect effect on Lyme disease risk and are important to monitor as they can become infected once they feed on an infected host and are potential threats in their advanced life stages. A chain reaction is observed as an abundance of larval ticks surviving and becoming infected increases the number of nymphal ticks the following year, which creates a larger adult peak in the fall. These seasonal and short lived peaks effect one's risk of encountering a tick and are followed by obvious declines. To address one's risk of contracting Lyme disease, it is essential to investigate what accounts for seasonal tick declines (Figure 1).

Diseases often trigger and are reflective of a collection of associated factors. The recent increase in Lyme disease incidences appears to be directly correlated with the number of host-seeking ticks in a given season. Since abundance in host-seeking ticks depends on seasonal tick declines, focus has been aimed toward the impacts of leaf litter and host availability on tick persistence. Seasonal tick declines, are caused by two factors, with opposing implications, death due to failure to obtain a host or survival due to encountering a suitable host. If declines in tick numbers can most greatly be attributed to deaths due to inability to find a host, risk of Lyme disease is reduced. However, if declines can be associated with attachment and presumably survival on a host, infection rates are likely to increase in the subsequent year.

Questing ticks seek out microhabitats with low temperature fluctuations and ample humidity, to increase survival rates until a sufficient host is encountered. To best satisfy these requirements, it has been documented that larval and nymphal ticks tend to prefer leaf litter, which supplies a suitable habitat for tick survival (Schulze et al., 2002). In contrast, dry conditions are less favorable as ticks are more susceptible to drying out. Dense areas of leaf litter are also suitable habitats for shielding small mammals from predators, and therefore are the areas most densely populated with small hosts. Larval and nymphal ticks favor small hosts, including white-footed mice, which possess the highest rate of transmitting *Borrelia burgdorferi*, the bacterium responsible for Lyme disease (Ostfeld 1997). Ticks depend on blood meals to survive and mature throughout their life cycle, as blood is their sole source of water, nutrition and energy. In light of these factors, leaf litter provides ticks with their preferred habitat and their preferred hosts, which increase the rate of encountering an infected tick.

Taking into account these two factors, one begins to observe how the environment and host availability are intertwined in aiding survival, tick density and ultimately in increasing one's risk of acquiring Lyme disease. It has been documented that as a consequence of reducing host reservoirs, Lyme disease risks were modified, justifying the significance of host populations on the intensity of risk (Ostfeld et al. 1995). Likewise, correlations between tick abundance and incidences of Lyme disease have been observed (Cartter et al. 1998). The goal of identifying what accounts for seasonal tick declines, under specific conditions, aims toward labeling areas and factors responsible for increased entomologic risks. Such findings may allow humans to take proper measures in avoiding and/or protecting themselves in such areas.

METHODS AND MATERIALS

Choosing sites

Maple dominated forest were preferred, as maple has demonstrated high tick densities for the majority of years, excluding masting, and possess constant dynamics in terms of tick/host availability (R.S. Ostfeld, unpublished data). These sites also consist of more open and less shrubby areas allowing focus on the environmental effects of leaf litter on tick survival. Aside from the dominant Sugar Maple, the forest also consisted of a few other tree species, including Black Cherry, Black Oak, and Shagbark Hickory.

Plot Preparation

To avoid interruption and stability of cages, flat independent forest areas were selected to conduct this experiment. The experimental treatments consisted of four different 1 meter radius plots (2m diameter), each of which were approximately 2-5 meters apart to maintain similar environments. There were four replicates, making a total of sixteen plots and these clusters of four were a minimum of 30 meters apart to assure independence amongst sites. Each site consisted of two caged plots, one with leaf litter intact and one with leaf litter removed (1m x 1m radius of 1.27cm mesh hardware cloth) and two un-caged plots, one with leaf litter intact and one with leaf litter removed (1m radius). To ensure no small animal entry into caged sites, a 2.5cm deep trench was dug to create an indentation were the cage was placed and buried into the ground. Through this we investigated the effects of two separate variables; leaf litter on survival and host availability on persistence (Tao et al. 2006) (Figure 2).

Preparing Sites for Tick Introduction

Each site was cleared of all pre-existing ticks by placing in the center of each plot two pounds of dry ice, in Styrofoam cups, covered with newspaper, to concentrate ticks towards the center. This was introduced approximately four hours prior to exhaustive dragging, which entailed dragging until no ticks were recovered after three consecutive attempts. This process is referred to as "zeroing" the plot, allowing the experiment to commence at a known sample size. An additional 1 meter radius was dragged, with $1m^2$ corduroy cloth, to create a buffer along the borders of the experiment. To ensure that the confined conditions inhibit small mammal entry and to verify that small mammals have indeed entered desired sites, animal track plates were utilized. A total of five track plates per site were placed within the radius of the sites and monitored several times weekly. Track plates in caged sites where monitored for a duration of a week due to difficulty in dismantling and reconstructing cages. The caged sites were accepted as successful, during this period of time, as no tracks were recovered inside the cages, while no caged sites had clear indications of visitation. Track plates were made by following a protocol consisting of a mixture of graphite, 90-100% anhydrous ethyl alcohol and mineral oil spread on 14 x 22 cm acetate sheets (Connors 2005).

Sampling Size and Tick Introduction

Both larval and nymphal ticks were collected by random dragging along the boarders of trails and tick dragging sites (44/Bacon Flats/ Fern Glen/Green House/Tea House) on IES grounds. Ticks were placed in glass vials with Plaster-of Paris base, stored in desiccators and refrigerated. A total of 640 ticks were collected, allowing introduction of 20 larvae and 20 nymphs to each site. Ticks were introduced to sites by laying the vials on their sides in the center of each site and left undisturbed for 30 days.

Recovery of Ticks and Data Analysis

Nearly the same method employed to zero the plots was performed to recover ticks. However, an additional 20 minutes of dragging within the 1m radius and 5 minutes along the buffer of the plot was conducted, followed by exhaustive dragging. Ticks collected were preserved in ethanol for later reference. Data collected was analyzed by two factor ANOVA (Figure 3).

RESULTS

In observing the number of larval ticks remaining after 30 days, there was no statistical significance in tick persistence between +Leaf litter (leaf litter intact) and -Leaf litter (leaf litter removed) (see Figure 4). Likewise, the effect observed between +Cage (no host) on tick numbers and – Cage (host access) was insignificant. There was no correlation between larval tick attrition and host availability or leaf litter treatments when analyzed simultaneous, concluding that no interaction between the two conditions was significant.

In regards to nymphal ticks +/- Leaf litter had no apparent association and no statistical significance on the number of nymphal ticks recovered (see Figure 5). In comparing +/- Cage there were approximately three times as many ticks in caged sites, where hosts were absent, compared to no caged sites, where host were allowed access (P=0.044). No significant interaction between host availability and leaf litter treatments were observed (Figure 4 and Figure 5).

DISCUSSION

No significant effects of leaf litter treatments on attrition rates for larval or nymphal ticks were detected. This outcome contrasts with observational studies suggesting that leaf litter enhances tick survival and abundance (Hung et. al. 2002). One possible reason for the discrepancy between the current study and others is that the protective value of leaf litter is reduced in closed canopy forests, where temperature and humidity are moderated by dense shade. Despite such findings, further investigation and expansion in sample size, to compensate for low statistical power would be beneficial and highly recommended. For nymphs, but not larvae, the exclusion of hosts significantly decreased losses. Such results imply that substantially more nymphal losses were due to attachment to a host, rather than death due to the environmental conditions present. Availability of hosts diminishes the number of host-seeking ticks, which causes an immediate decrease on Lyme disease risk. However, due to the increased probability of those ticks surviving to their subsequent life stage, Lyme disease risk is heightened in the succeeding stages. Future research should quantify the net effect of decreasing tick-host encounter rates on current and future Lyme disease risk to humans. The interactions between host availability and leaf litter treatments were not significant for either life stage, suggesting that leaf litter had only negligible effects on behavior of small-mammal hosts. Again, a larger study would increase statistical power.

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APPENDIX

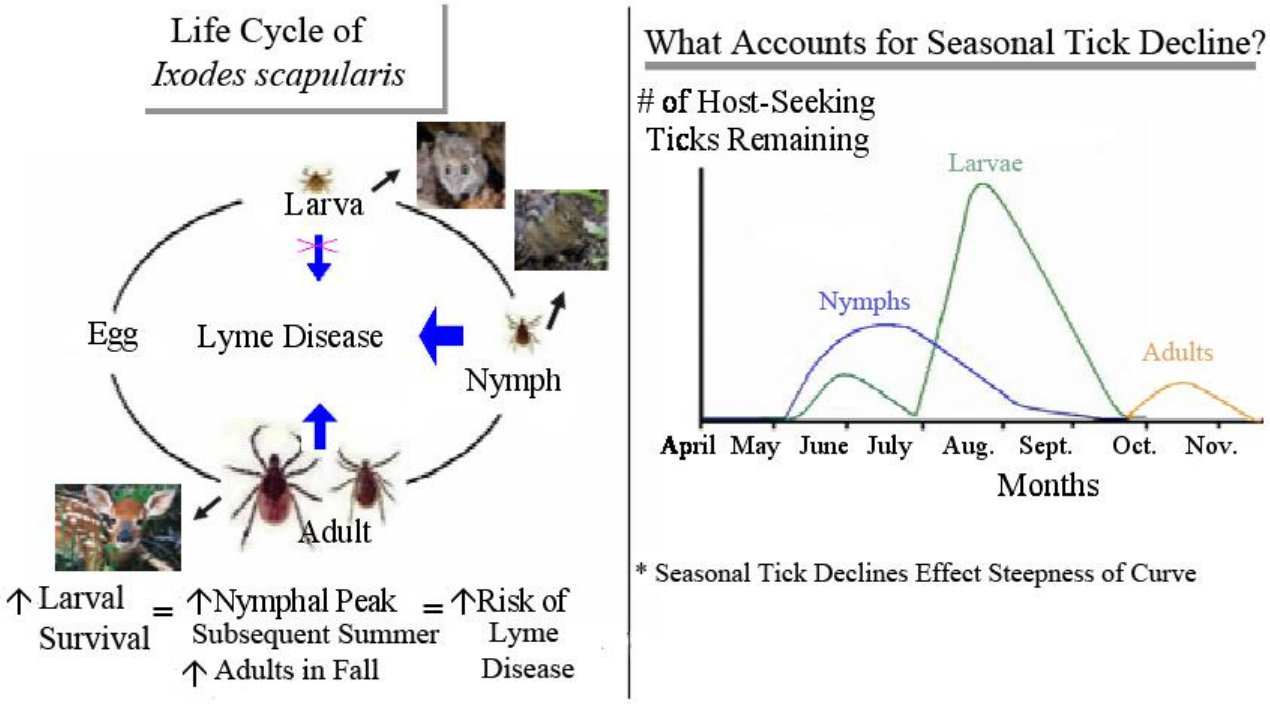
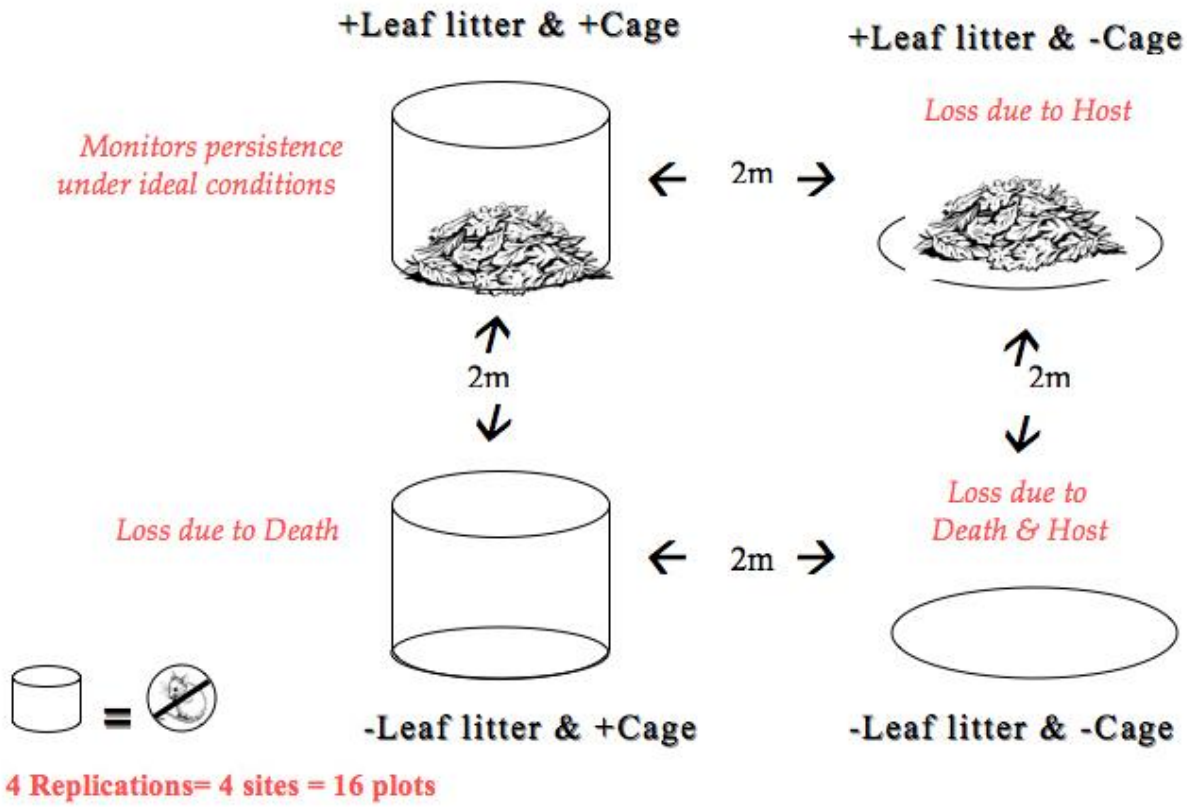


FIGURE 1: (left) Diagram of the tick life cycle. Indicates the preferred host at each specific life stage and impact each active life stage has on Lyme disease risk. (right) Diagram depicts the seasonal peaks and declines of the three active tick life stages

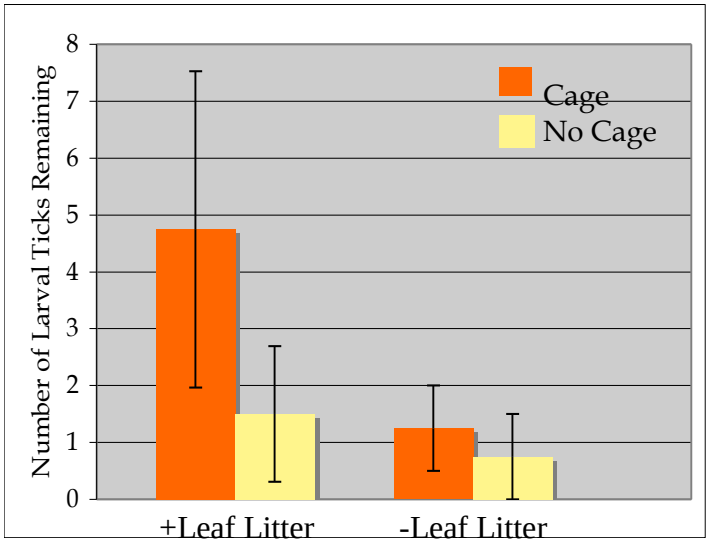


Four Treatments Under Investigation:

1. + Leaf litter/ + Cage
2. + Leaf litter/ - Cage
3. - Leaf litter/ + Cage
4. - Leaf litter/ - Cage

FIGURE 2: Blueprint of experimental design and a brief description of what each plot investigates. Represented is one site, which consists of four distinct treatments or plots.

FIGURE 3: This photograph captures 1 experimental site, consisting of four plots in a closed canopy forest. In total 4 sites were utilized, approximately 30 meters apart from one another.



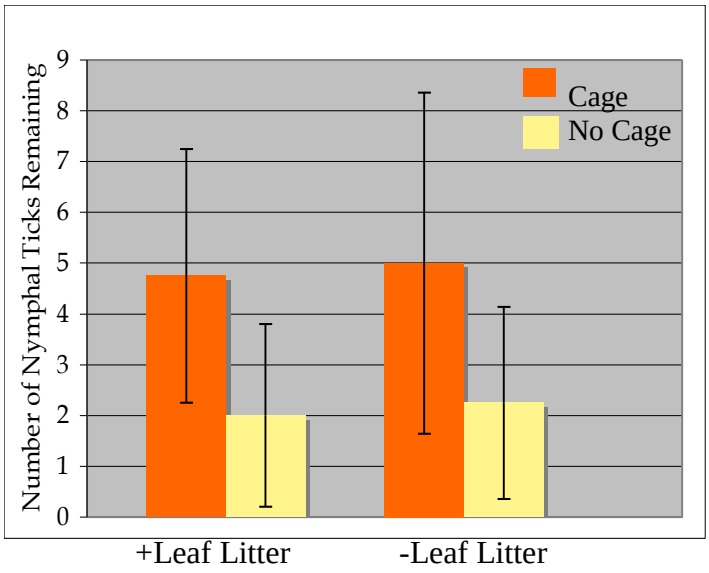
Leaf Litter P= 0.173

Cage P = 0.165

Interaction P= 0.501

- Low Statistical Power:

FIGURE 4: Data on the number of larval ticks remaining in experimental treatments suggested that there was no statistical significance of either the cage (host removal) or leaf litter removal on the numbers of larvae recovered after 30 days. No statistically significant interaction between the cage and leaf litter treatments was observed.



Leaf Litter P= 0.841

Cage P= 0.044

Interaction P= 1.00

FIGURE 5: Data on the number of nymphal ticks remaining in experimental treatments suggested that there was no statistical significance of leaf litter removal on the numbers of nymphs recovered after 30 days. The removal of hosts significantly increased the number of nymphs recovered in comparison to sites where hosts were allowed access. No statistically significant interaction between the cage and leaf litter treatments was observed.