CAN AN INVASIVE HERB AFFECT LYME DISEASE RISK? EXAMINING THE INTERACTIONS BETWEEN GARLIC MUSTARD, ENTOMOPATHOGENIC FUNGI, AND BLACKLEGGED TICKS

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Abstract. The density of blacklegged ticks (*Ixodes scapularis*) infected with the Lyme disease spirochete (*Borrelia burgdorferi*) determines the Lyme disease risk in a given area. Thus understanding processes that affect tick densities has important human health implications. Entomopathogenic fungi are known to cause tick mortality, but have been shown in lab experiments to be inhibited by garlic mustard (*Alliaria petiolata*), an invasive herb common to many Lyme disease endemic areas. This study investigated how garlic mustard affects tick mortality indirectly through the inhibition of entomopathogenic fungi. In a bioassay using pathogen-sensitive wax moth larvae (*Galleria mellonella)* as bait, wax moth larvae placed in plots with garlic mustard had significantly lower rates of fungal infection than did those placed in plots without garlic mustard (p=0.047). Overall, 11.9% of blacklegged ticks became infected with entompathogenic fungi. Engorged nymphal blacklegged ticks did not exhibit a significantly higher fungal infection rate between garlic mustard treatments (p=0.250). There was no significant difference in overall blacklegged tick mortality in plots with (18.9%) and without (36.9%) garlic mustard (p=0.094). However, that overall tick mortality was nearly twice as high in garlic mustard plots suggests that secondary compounds found in garlic mustard exudate may have direct lethal effects on blacklegged ticks that outweigh those of entomopathogenic fungi. While entomopathogenic fungi are known biocontrol agents for ticks, garlic mustard itself may play a prominent role in controlling natural tick densities where it is present. Further study is warranted to investigate the effects of garlic mustard exudates on blacklegged tick molting success.

INTRODUCTION

Lyme disease is the most common vector-born zoonotic disease in North America, with an increasing prevalence in the past two decades (Bacon et al. 2008). Transmission of the Lyme disease bacteria (*Borrelia burgdorferi*) to humans requires a minimum of four species: the human victim, the Lyme disease pathogen *Borrelia burgdorferi*, the vector (blacklegged ticks, *Ixodes scapularis*), and a wildlife reservoir (Ostfeld et al. 2006a). While the mechanism of human infection between the vector, pathogen, and humans is well understood, less is known about the interactions between ticks, *B. burgdorferi* host reservoirs, and the physical environment. Thus, understanding the community ecology of Lyme disease is crucial to understanding human disease risk. Local density of blacklegged ticks infected with *Borrelia burgdorferi* is the most important determinant of Lyme disease risk in a given area (Ostfeld et al. 2001). A variety of environmental factors influence both tick densities and infection prevalence. Composition of the host community is strongly related to infection prevalence, as certain species such as white-footed mice (*Peromyscus leucopus*) are often locally abundant and particularly competent reservoirs for the Lyme disease spirochete (Keesing et al. 2006, Ostfeld et al. 2001, Ostfeld and Keesing 2000). Increased diversity in the host community "dilutes" the pool of highly competent hosts, so biodiversity in vertebrate hosts is an important ecosystem function in influencing tick infection prevalence (Ostfeld and LoGiudice 2003).

Additional environmental factors that affect tick populations also play an important role in Lyme disease risk. Of a suite of factors considered, weather-related variables fail to consistently predict Lyme disease incidence, but oak tree acorn production and the subsequent response of small mammal populations is positively correlated with Lyme disease incidence (Schauber et al. 2005). However, other factors are likely at play as well, particularly in non-oak dominated landscapes. Another environmental factor capable of influencing tick populations is the presence of entomopathogenic fungi. Two species in particular, *Beauveria bassiana* and *Metarhizium anisoplae*, have near worldwide distribution (Bidochka et al. 1998) and are known to significantly reduce blacklegged tick fitness in both field and lab experiments (Benjamin et al. 2002, Hornbostel et al. 2004). As a result, they are touted as potential biocontrol agents for ticks. However, the effectiveness of entomopathogenic fungi as an acaricide is dependent on several factors including the life stage of ticks, whether they are flat or engorged, and the environmental conditions. Blacklegged tick mortality due to fungal infection is higher in engorged ticks than flat ticks, and also in lab conditions that are favorable to fungal growth (Ostfeld et al. 2006b).

Although entomopathogenic fungi show the potential to limit natural tick populations, the growth of the fungus itself may be inhibited by environmental factors. Garlic mustard, an invasive biennial herb of Eurasian origin, exudes secondary compounds that not only deter herbivory but are capable of disrupting the growth arbuscular mycorrhizal fungi when leeched into the soil (Rodgers et al. 2008, Stinson et al. 2006). This provides a mechanism for its ability to outcompete native plants and fungi, even in old growth forests, thus reducing species richness where it is present (Meekins and McCarthy 1999). Importantly, garlic mustard has also been shown to inhibit growth of the entomopathogenic fungi *Beauveria bassiana*, a known pathogen of blacklegged ticks (Keesing et al., unpublished manuscript). Thus, by inhibiting growth of a natural tick control agent, garlic mustard may be indirectly increasing *I. scapularis* abundance.

While certain entomopathogenic fungi show potential as biocontrol agents for *I. scapularis*, our understanding of the role entomopathogenic fungi play in controlling natural tick abundance is lacking. Further, inhibition of entomopathogenic fungal growth by garlic mustard, and the impact of garlic mustard on overall community ecology, may have important implications for *I. scapularis* density. This study investigated the effects of garlic mustard on the survival of engorged nymphal *I. scapularis* in a field setting by placing ticks in forest plots with and without garlic mustard, and in which entomopathogenic fungi is known to be present and reporting fungal infection rates and mortality. It was hypothesized that in natural forest communities garlic mustard would decrease tick mortality by inhibiting the growth of entomopathogenic fungi, a known pathogen of blacklegged ticks.

METHODS

Site Selection

Field studies were conducted on the property of the Cary Institute of Ecosystem Studies in Dutchess County, southeastern New York. Test sites were located in postagricultural forest lands with a primarily mixed oak/maple canopy (Ostfeld et al. 2001). Eight 2x2m plots covered in garlic mustard were selected across the property. For each garlic mustard (GM+) plot, a corresponding 2x2m garlic mustard absent (GM-) plot was selected nearby, no more than 20m from GM+ plots. Because sunlight reaching the forest floor is an important influence in plant performance, species composition, and soil characteristics, all potential sources of variability between paired plots, light analysis in each plot was performed. Total percent diffuse and direct solar transmission reaching each plot was measured using fish eye photography and Gap Light Analyzer software. Potential sites with light transmission that differed +/- 2 Std. deviations from the mean were not used in the study.

Mouse and Chipmunk Trapping and Rearing

Mice and chipmunks were live caught using Sherman traps from approved trapping grids on the Cary Institute property and greater Dutchess County. Animals were kept in wire mesh cages suspended over plastic tubs, fed twice daily, and kept on a consistent light/dark cycle. Animals were kept for no longer than three days, before being released to the exact location from which they were trapped.

Tick Collection

Mouse and chipmunk cages were suspended over plastic tubs with damp paper towel in the bottom. Double sided mounting tape was placed around the top of tubs to prevent tick escape. After falling to the paper towels at repletion, ticks were collected, placed in damp plaster-bottomed vials, and stored in a dark drawer at room temperature until field deployment.

Galleria Bait Method

Wax moth *(Galleria mellonella)* larvae were used as a bioassay to indicate the presence of entomopathogenic fungi in each potential plot. 1 tsp. of microsite topsoil and 4 *G. mellonella* were placed in organdy mesh bags in 3 microsites per plot. The open end of organdy bags was folded multiple times and stapled to prevent escape. Because engorged nymphal ticks naturally fall to repletion on soil and leaf litter, and topsoil has the highest natural abundance of entomopathogenic fungi (Tuininga et. al 2009), bags were placed on topsoil and covered in leaf litter, then covered in ¼ inch wire mesh cages anchored with landscaping staples for protection from animal disturbance. After 11 days *G. mellonella* were retrieved and checked for fungal growth. Fungal infection of at least one *G. mellonella* per bag indicated the presence of entomopathogenic fungi for that microsite.

Tick Field Deployment

Directly adjacent to each *G. mellonella* bag, one engorged nymphal-blacklegged tick was placed in an organdy bag with 1 tsp. of topsoil, the bag was sealed, and covered in leaf litter and protective mesh caging. Tick bags were left undisturbed for 25 days, from July 15th-August 3rd, then placed individually in labeled, humidified, plaster-bottomed vials, and brought to the lab for assessment.

Tick Assessment

Indoors, organdy bags were opened and microsite soil was sifted to locate ticks. Ticks from all sites were assessed for mortality, sex, and life stage and/or molting status using a dissecting microscope. Mortality of ticks with visible fungal mycelia was considered to be the result of fungal infection. Unmolted ticks were allowed additional days in humidified glass vials at room temperature to determine survivorship. Within 5 days of field collection, all ticks had either molted into live adults or failed to molt, with visible ruptures in the dorsal exoskeleton.

Statistical Methods

Fungal infection for waxworms was tested using a paired t-test for difference in means. Fungal infection and mortality in ticks was tested using a non-parametric Mann-Whitney U-test for difference in means. Because the a priori expectation was that garlic mustard would decrease tick fungal infection rates, a one-tailed test statistic was used.

RESULTS

Galleria Bait Method

In all, 192 *G. mellonella* were deployed in the field across eight study sites. *G. mellonella* had a 100% mortality rate after 11 days in the field. Entomopathogenic fungi were isolated on *G. mellonella* in 6 (75%) in GM+ plots and 7 (87.5%) of GM- plots ($p=0.047$). A total of 47.9% and 66.8% of *G. mellonella* deployed showed visible fungal mycelia in GM+ and GM- plots, respectively (Figure 1).

Tick Fungal Infection

8 ticks were never recovered, including all three from one GM- plot, resulting in n=19 for GM- plots and n=23 for GM+ plots. Overall, 11.9% of blacklegged ticks exhibited growth of fungal mycelia across all sites, establishing an estimate of overall blacklegged tick offtake due to fungal infection in natural conditions. However, only 1 (5.3%) tick in GM+ plots showed sign of fungal infection, while 4 (17.4%) showed sign of infection in GM- plots. There was no significant difference in fungal infection between plots with and without garlic mustard (p=0.250), even though nearly four times as many ticks became infected in GM- plots (Figure 2). Blacklegged ticks exhibited fungal mycelia in only 25% of plots in which entomopathogenic fungi was isolated on corresponding *G. Mellonella* bags.

Tick Mortality

Overall, 13 (30.9%) nymphs did not molt successfully after 30 days. Mean site mortality was 37% and 19% for GM+ and GM- plots, respectively. There was no significant difference (p=0.094) between overall mortality in GM+ and GM- plots (Figure 3).

DISCUSSION

While the survival and distribution of *Ixodes* ticks is subject to a near-infinite number of environmental factors, for the purpose of study one must prioritize interspecies interactions that most strongly affect the fate of ticks, and by extension human disease risk. This study sought to isolate the interactions between blacklegged ticks, entomopathogenic fungi, and garlic mustard. Because waxworms are highly susceptible to fungal infection, they were utilized to determine where entomopathogenic fungi were present in the study plots. Waxworms had a higher incidence of fungal infection in GM- plots, indicating that garlic mustard was likely inhibiting entomopathogenic fungi growth ($p<0.05$). This effect has previously been observed in controlled lab conditions (Keesing et al., unpublished manuscript) and is now confirmed in a field investigation. By inhibiting the growth of a natural pathogen of arthropods, garlic mustard may be altering the abundance and distribution of insect species in areas where it is present. The trophic ramifications of this effect are potentially wide-reaching, and could be a subject of future study.

Overall, 11.9% of ticks failed to molt and had visible mycelia on the cuticle 28 days after field deployment. This represents a baseline estimate for nymphal blacklegged tick mortality due to fungal infection. Thus, entomopathogenic fungi play a key role in controlling tick molting success in a natural environment. Since waxworms contracted fungal infection more frequently in GM- sites, it was predicted that ticks would also exhibit higher infection rates in plots without garlic mustard. This was not the case, however. Despite the fact that fungal infection rate was four times higher in GM- plots, the quantity of ticks that developed fungal infection was not large enough to yield a robust statistical difference. If the trend held, a larger sample size would demonstrate the inhibitive effect of garlic mustard on entompathogenic fungal infection rates for blacklegged ticks.

It should be noted that although all ticks in this study with visible fungal mycelia failed to molt successfully, tick mortality in this study cannot definitively be attributed to fungal infection. Benoit et al. (2005) reports that blacklegged ticks can handle heavy surface fungal loads without apparent detrimental effects or changes behavior. Thus, although 11.9% of ticks showed signs of fungal infection, this figure is likely an overestimation of the rate that ticks actually died from fungal pathogens. Isolating and identifying fungal species found of exterior of sampled ticks would have shed light on this issue, as it appears that only select fungal species can cause mortality in ticks (Benoit et al. 2005, Ostfeld et al. 2006).

While the study sought to investigate an indirect relationship between blacklegged ticks and garlic mustard via the effects on fungal infection rates, the results suggest a potential direct effect of garlic mustard on blacklegged ticks. Molting failure was nearly twice as high for ticks in GM+ plots. While the result did not meet the standard for statistical significance (p=0.094), the result is suggestive of a direct lethal effect of garlic mustard exudates on tick molting success. Garlic mustard secondary compounds include glycosinolates, which hydrolyze into cyanide compounds, which are known to disrupt the respiratory election transport in a variety of organisms (Rodgers et al. 2008). Garlic mustard secondary compounds have been shown to be toxic to soil borne plant pests (Brown and Morra 1997). Engorged nymphal ticks in this study, who were directly exposed to soil where garlic mustard was growing, were highly likely to come into contact with these compounds.

Although the results are not conclusive, the overall tick mortality suggests that garlic mustard exudates may have a direct role in reducing tick molting success from the nymphal to adult stage. Garlic mustard is known to have multiple deleterious effects on native forest species, and blacklegged ticks may be no exception. Future study into the direct impact of garlic mustard secondary compounds on blacklegged tick survival is warranted to determine if garlic mustard is indeed a key factor in tick molting success. While invasion by garlic mustard and other nonnative species is commonly associated with the loss of ecosystem services, in this instance garlic mustard may be reducing populations of *Ixodes scapularis*, a species with a profound negative impacts on human health.

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APPENDIX

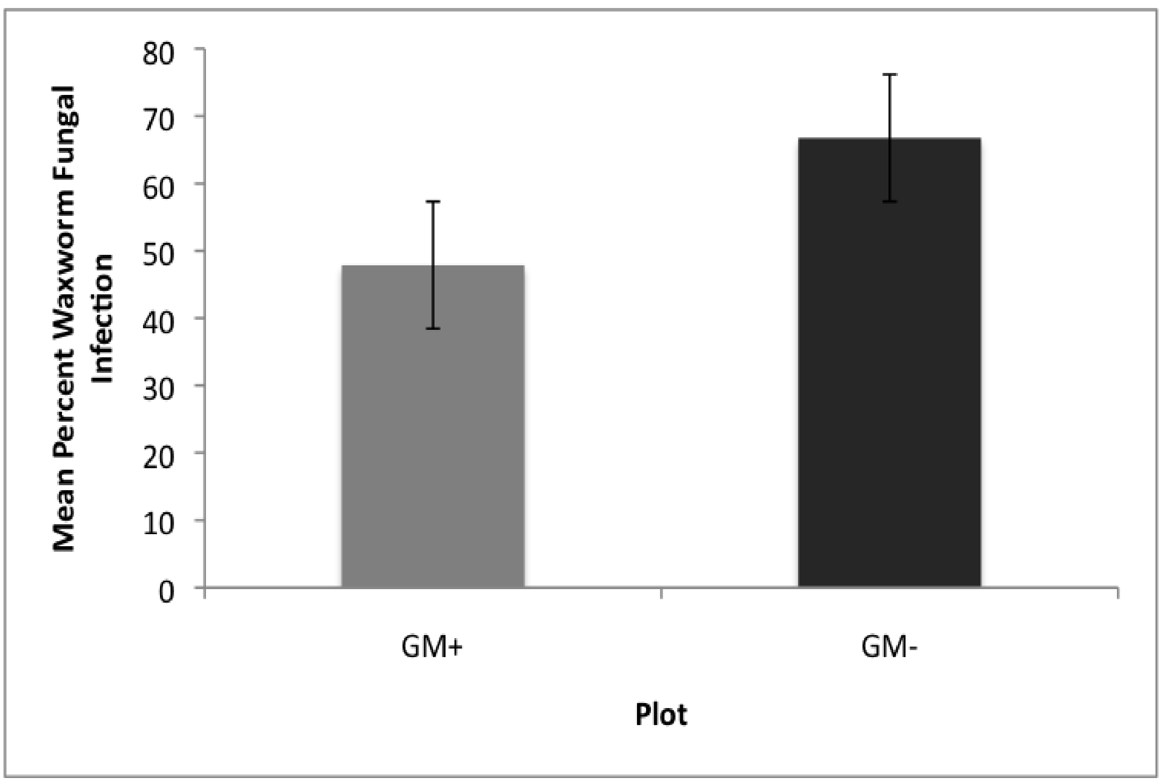

FIGURE 1. Mean waxworm fungal infection for GM+ and GM- plots 11 days after field deployment (n=96, p=0.047).

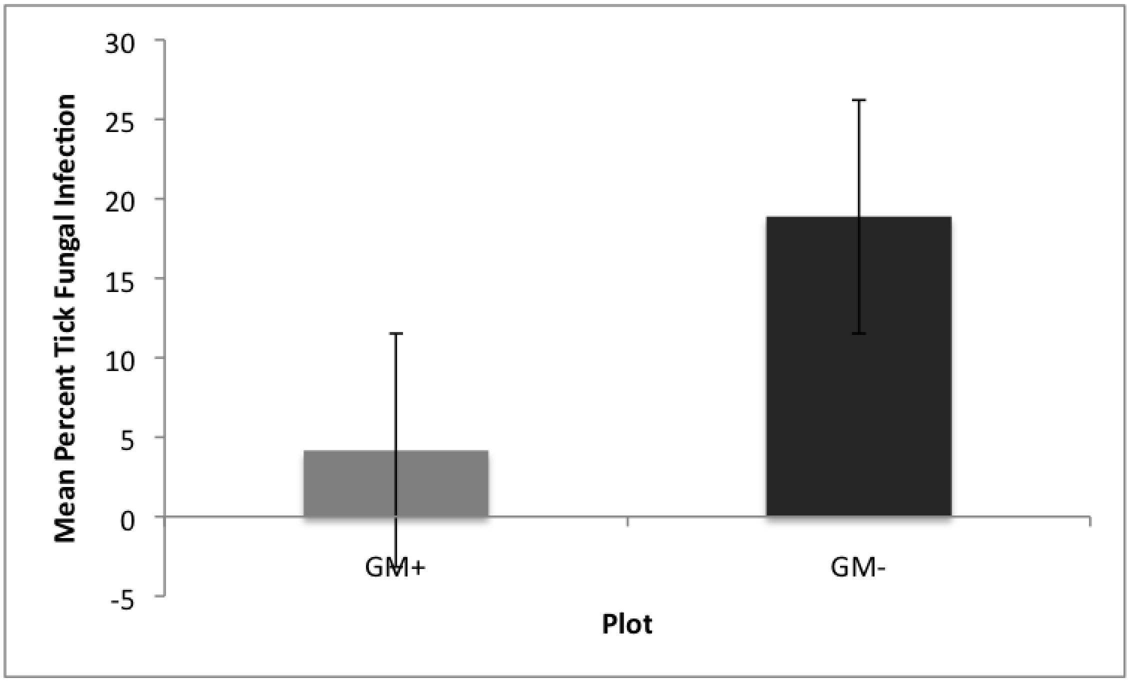

FIGURE 2. Mean percent blacklegged tick fungal infection in GM+ and GM- plots 28 days after field deployment (n=22, p=0.250).

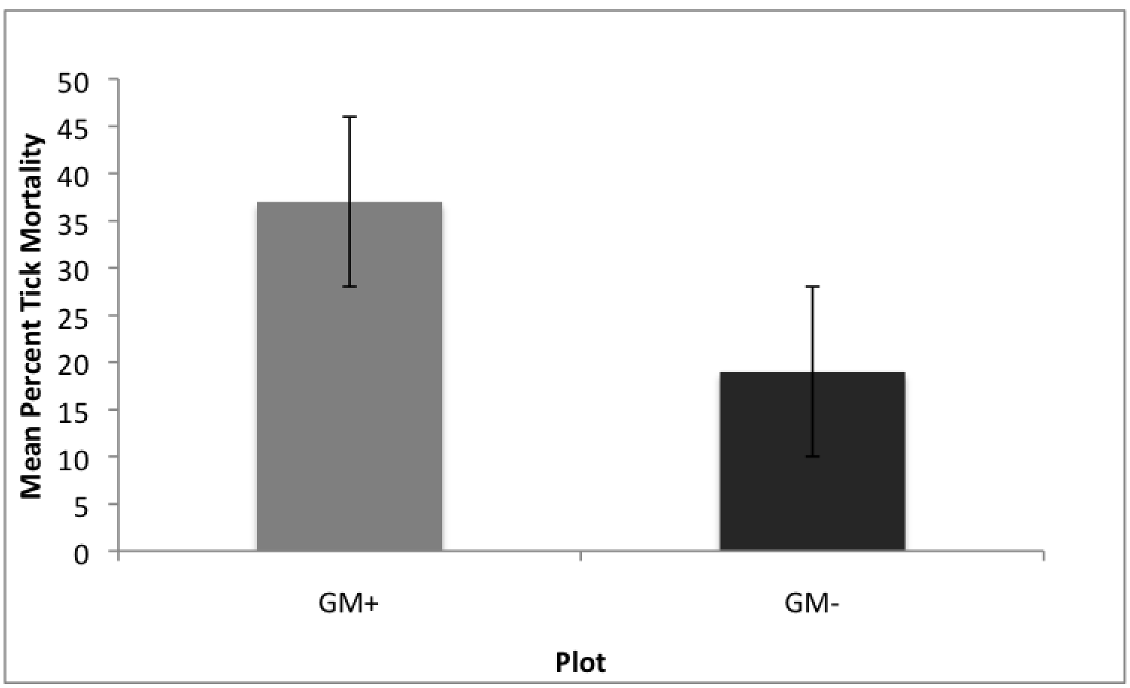

FIGURE 3. Mean percent tick mortality in GM+ and GM- plots 28 days after field deployment (n=43, p=0.094).