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Lethal and Sublethal Effects of Beauveria Bassiana on Maine Ticks Across Soil pH

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LETHAL AND SUBLETHAL EFFECTS OF BEAUVERIA BASSIANA ON MAINE

TICKS ACROSS SOIL PH

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ABSTRACT

Ticks are obligate parasite arthropods that are becoming increasingly common in northern regions of the United States. Ticks such as the black-legged tick (*Ixodes scapularis*) and the American dog tick (*Dermacentor variabilis*) are vectors for pathogens that cause a wide range of diseases, and as these ticks increase their exposure to humans, the diseases they transmit become more prevalent. This upward trend in cases of tick-borne illnesses has necessitated the pursuit of tick control methods that can be used across the diverse environments that are present in tick habitat ranges. One such control method is the fungal biological control, *Beauveria bassiana*. This study investigates the effects of soil pH on *B. bassiana* as a tick control method, focusing on its effects on tick mortality and questing behavior. These variables were measured for *I. scapularis* and *D. variabilis* in a laboratory bioassay across soils with pH 6.5 and pH 5.4. No significant difference in mortality was observed between soil treatments without *B. bassiana*. The only significant difference in questing behavior was observed for *I. scapularis* nymphs between soil treatments without *B. bassiana*, which saw an increase in questing in more acidic soil. Difference in soil pH was not found to have a significant effect on *B. bassiana* and its effect of increased mortality on ticks. *Beauveria bassiana* application led to a significant increase in tick questing for all tick species and life stages in soil with pH 6.5. Application of *B. bassiana* was found only to significantly decrease questing behavior for *I. scapularis* adults in soil with pH 5.4.

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INTRODUCTION

Ticks are arthropods that are obligate parasites of many animals, including mammals, reptiles, birds, and some amphibians (Anderson and Magnarelli 2008). The pathogens carried by ticks and the diseases they cause make up over 90% of vector-borne disease cases in the United States (Eisen et al. 2017). Two medically important ticks in North America are the black-legged tick, *Ixodes scapularis*, the vector for the pathogens that cause Lyme disease (*Borrelia burgdorferi*; Burgdorfer et al. 1982) and human babesiosis (*Babesia* spp.; Skrabalo and Deanovic 1957), and the American dog tick, *Dermacentor variabilis*, the vector for Rocky Mountain Spotted Fever (*Rickettsia rickettsii*; Wolbach 1916) (Madison-Antenucci et al. 2020). Globally, billions of dollars are lost annually with livestock deaths, controlling tick populations in livestock settings, and the contamination of products from these control measures (Beys-da-Silva et al. 2020). Billions more are lost each year in medical treatment of human illness caused by tick-borne diseases and years lost in life. These costs are enough to justify research in tick control and management practices (Mac et al. 2019).

Ticks can be found throughout the world but are moving further north and south, facilitated by the rising global temperatures, expanding ranges of tick hosts, and changes to habitats (Ogden and Lindsay 2016; Ogden et al. 2008, Medlock et al. 2013). In the Northeastern United States, human encounters with ticks have increased dramatically since the 1980's (Smith et al. 2019). Tick populations are highest in wooded areas but can be encountered in parks, fields, and lawns (Wickings et al. 2015, Sormunen et al. 2020). Cases of Lyme disease, the most common tick-borne disease, have been increasing over the past decades as tick habitat range has expanded and human exposure to ticks has increased (Hofhuis et al. 2015). In Maine, Lyme disease diagnoses have doubled in the past ten years to over 2,500 reported cases in 2022, but this is likely an underestimate of the true number due to many cases of Lyme disease going unreported (Maine CDC 2023, Meek et al. 1992).

As the danger posed by ticks and the diseases they can transmit has increased, so has the public health importance of preventing such diseases. Some examples of preventive efforts include personal protective habits, removing hosts, forest management, vaccination, and pesticides. Personal protection habits, like tucking pants into socks and daily tick checks, are the easiest to carry out and are suggested to be effective preventions of contracting Lyme disease (Orioski et al. 1993; Ley et al. 1995). However, while these practices may protect an individual from exposure to tick-borne pathogens, they do not reduce overall densities of ticks in the environment. Removal of important hosts, such as white-tailed deer (*Odocoileus virginianus*), has been demonstrated to be quite effective but is only feasible on some isolated islands where these hosts cannot be reintroduced, such as Monhegan, Maine (Rand et al. 2004). Research looking at excluding white-tailed deer from localized areas has had varying and inconclusive results. For example, Stafford (1993) found that deer enclosures reduce tick presence by about 50% across two years, while Perkins (2006) found that smaller deer enclosures led to an increase in tick abundance as well as an increase in more reservoir-competent hosts. Changes to forest landscape have been explored with timber harvesting and other forest management, which seem to reduce tick presence, but this is physically and financially demanding and only useful in private use with larger plots of land (Williams et al. 2009; Padgett et al. 2009; Conte et al. 2021). A vaccine for Lyme disease was introduced in 1998 but was removed only four years later. Although it was 75% effective at preventing Lyme disease it was not commercially successful, and there was concern that people would stop other preventative measures like tick checks, increasing their risk of contracting other tick-borne diseases (Hayes and Piesman 2003). Chemical acaricides are quite effective and have been used extensively as a preventative measure for humans and livestock (Mount and Snoddy 1983; Connally et al. 2018), but environmental concerns about their overuse are growing, and acquired resistance among tick populations continue to reduce their effectiveness (George 2006). Thus, there is a need for a strategy that is effective at controlling ticks and reducing the spread of disease, financially and physically accessible, and causing minimal environmental damage and disturbance.

An emerging tool that is of increasing interest in the fight against ticks are biological controls (hereafter 'biocontrols'), which are organisms that are natural enemies that kill or incapacitate ticks. Biocontrols have long been investigated as a tick control, as with bacteria like *Rickettsia* (Friedhoff 1970), nematodes (Kocan et al. 1998), predators (Stutterheim et al. 1988; Lavalle 1923), and fungi (Castineiras et al. 1989). Entomopathogenic fungal biocontrols are especially attractive due to their selectiveness in the species they target, reduced potential for environmental and human harm, genetic variety, which increases the likelihood of finding more effective strains, and ability to evolve with their hosts, reducing the potential for ticks developing resistance (Samish and Rehacek 1999). In the US, fungi of the genera *Metarhizium* and *Beauveria* have been investigated the most for tick control, with both demonstrating high mortality rates among many species of ticks (Kaaya et al. 1996; Arthurs and Dara 2019). Both are found in soils across the globe and have been observed to be pathogenic to ticks in nature

(Brownbridge et al. 1993; Sullivan et al. 2020). These fungi infect ticks when ticks ingest fungal spores, or by contacting and penetrating the hemocoel of the tick by fungal structures. Once infected, a tick becomes sluggish, uncoordinated, and often paralyzed until death (Ferron 1978, Mora et al. 2018). While formulations for these fungal biocontrols have not been able to reach the level of efficacy of chemical acaricides, efforts are being made to increase potency and incorporate them into integrated pest management strategies (Fernandes et al. 2012).

Environmental conditions can impact the efficacy and virulence of entomopathogenic fungi as they do arthropods, like ticks. Increased soil moisture content for soils containing *B. bassiana* has been associated with higher tick mortality (Zhioua et al. 1999). Soil pH levels can also affect entomopathogenic fungi. Quesada-Moraga et al. (2007) and Groden and Lockwood (1991) both found that *B. bassiana* prefers more neutral pH soils, but no research looking at how soil pH affects the effectiveness of *B. bassiana* as a tick biocontrol has been carried out. Despite numerous studies on the effects of entomopathogenic fungi on tick mortality, none have investigated its effects on questing behavior. Some tick species, especially those that are clinically significant, spend much of their life dormant in leaf litter and the top layers of soil but require a blood meal from a host–including humans–to progress to the next life stage (Needham and Teel 1986). Ticks will quest to find a host, which is when they climb a nearby tall object and extend their legs, waiting for a host to pass (Randolph 2004). Although this relationship between tick questing behavior and *B. bassiana* has not been investigated, *B. bassiana* has the potential to cause behavioral change, as observed in other arthropods (Manoussopoulos et al. 2019; and Roy et al. 2006).

As with other environmental factors, soil is an important variable in the microenvironment of ticks and plays a key role in the terrestrial habitat. The variations in soil have impacts on the life that it supports, from changes in soil moisture to heavy metal ion levels (Singh 1947; Ulrich et al. 1980; Singh and Kalamdhad 2011). Differences in soil pH and acidity lead to many chemical changes within the soil, namely altering the availability of aluminum ions, calcium ions, and salt concentrations (Thomas 1996). These changes strongly influence both the plant and microbial inhabitants (Childs and Hanks 1975; Zhalnina 2014; Soriano 2012). Maine soil pH values range from 4.5 to 6 but can be closer to 7 on farms where soil is limed and fertilized (Rourke and Beek 1968). Prior research indicates behavioral differences in arthropods in different soil pH levels, suggesting a connection between the two (Sastrodihardjo and Straalen 1993; Zimmer et al. 2000). Although no research looks at this relationship in ticks, the wide range of arthropods affected by soil pH suggests ticks may be vulnerable to variations in soil pH.

The primary objective of this work is to examine and identify any differences in the effectiveness of fungal biocontrols as a tick control strategy in different soil pH levels. As part of this, I tested the hypotheses that 1) soil pH will have an effect on the effectiveness of *B. bassiana* as a tick control, 2) *B. bassiana* will decrease the questing behavior of the ticks used, and 3) soil pH will have an effect on tick survival or questing behavior. The results from this will allow us to better understand one of the many environmental factors that impact the relationship between fungal biocontrol and ticks, as well as the implications of use across differing pH levels in soils.

METHODS

Bioassays

To test the hypothesis that differences in soil pH have no effect on the efficacy of the fungal biocontrol *Beauveria bassiana* in tick control, I conducted an experiment using 56 individual container units with different fungal biocontrol and soil pH treatments. A total of 280 ticks (95 *Ixodes scapularis* adults, 95 *Ixodes scapularis* nymphs, and 90 *Dermacentor variabilis* adults) were used across the experiment with 5 ticks in each unit. The units were split into two soil pH treatments (i.e., pH of 5.4 or pH of 6.5) and two fungal biocontrol treatments (i.e., fungal biocontrol present or fungal biocontrol absent, acting as a control). This yields a total of four treatment combinations: a pH 5.4 soil with and without fungal biocontrol and a pH 6.5 soil with and without fungal biocontrol. Overall, each treatment combination encompassed 70 ticks among 14 container units.

The container units were kept in a Darwin Chambers growth chamber, which controlled temperature (Figure 1). The ticks used in this experiment have activity rates highest at temperatures of 20℃ and 30℃ (Fieler et al. 2021), so a temperature of 25℃ was used. A Tempo Disc was placed inside the growth chamber to verify temperatures and record relative humidity (RH) levels. Due to the location of the thermostat within the growth chamber, the real temperature experienced by the ticks was around 24°C throughout the duration of the experiment.

Relative humidity is another condition that affects tick survival and activity (Vail and Smith 1998). Ticks survive best at a RH of around 85% or greater (Stafford 1994) and RH levels below 83% have negative effects on tick survival (Rodgers et al. 2007). As the growth chamber did not control humidity, the RH was kept at 100% throughout the experiment. This was achieved by placing two trays filled with water underneath the growth chamber fans which also shielded the container units from constant and direct airflow that would have dehydrated the ticks. The growth chamber remained dark during the experiment, and the only exposure to light came when the container units were removed for mortality and questing checks. It was necessary to conduct this experiment in a lab setting due to it taking place over the winter.

Figure 1. The setup of the 56 container units inside the growth chamber. At the top of the chamber are the pans of water for humidification. The blue Tempo Disc on the bottom shelf recorded temperature and humidity, which was moved to different spots to verify all units experienced consistent conditions.

Figure 2. An example of an individual container unit.

Each individual unit was a 500ml Rubbermaid TakeAlong, 3inx3in, square food storage container (Figure 2). Dried soil was added to each in an amount of 100g ±1g with 25 ml of deionized (DI) water added to moisten the soil. The fungal biocontrol (described below) was added in a water suspension, adding an additional 5 ml of DI water with the fungus. In the control units without fungal treatment 5 ml of DI water was added without the fungus. Research suggests that ticks prefer a moderate soil moisture content, at least greater than 15% (Hoch et al. 1971, Greenfield 2011), which is roughly measured as being moist to the touch. The 30 ml of initial water added to the soil achieved a soil moisture content of about 30%. Water was added to each unit with a dropper as needed during the experiment to maintain soil moisture between 15% and 30%, keeping the soil moist to the touch. To provide a questing surface, four bamboo sticks were placed an equal distance apart in each unit, each with a height equal to the container. When stored, each unit was covered with a breathable material to allow for oxygen exchange. A 0.7 oz MONOLITE™ Ripstop Nylon Mesh was used; squares of mesh were placed over the tops of each container tightly secured by a rubber band to prevent the ticks from escaping.

Ticks

The ticks used in this experiment were lab reared and sourced from the CDC. Current, unpublished research shows no difference in questing behavior between lab reared ticks and those collected from the wild. The tick species used were *Ixodes scapularis* and *Dermacentor variabilis*. These were chosen due to their medical importance in being vectors for diseases such as Lyme disease, anaplasmosis, and babesiosis. The adult life stages of both species were used as well as *I. scapularis* nymphs, which are smaller and harder to detect on skin, making them more clinically significant (Gray et al. 2002).

To measure the lethal effects of the fungal biocontrol, tick mortality was assessed and recorded every three days for the first 21 days, then again on day 28. The interval of three days was chosen due to previous similar research (Sullivan et al. 2020) and the high time cost of the mortality checks. Dead ticks were confirmed through visual microscope analysis and by submerging them in water to check for reactivity, then discarded.

Figure 3. A tick questing atop one of the bamboo sticks with its front legs extended.

Measuring the sublethal effects of the fungal biocontrol was achieved by recording the number of ticks questing for each container unit. In this experiment, a tick was defined to be questing whenever not on or in the soil (i.e., on the bamboo sticks, on the sides of the container, or on the mesh covering) (Figure 3). Questing behavior was checked every day for the first 21 days, then again on day 28. Ticks can sense and move towards nearby carbon dioxide sources (Petney et al. 2011) so container units were removed from the growth chamber and checked one at a time to accurately measure the questing behavior. Once the questing checks were complete the ticks were gently placed on top of the soil using forceps. This was done in order to reset them to a non-questing state to ensure they were questing again at the next check, as well as to increase the exposure to the fungal biocontrol that was present in the soil.

Soil

The soil used in this experiment was sourced from Rogers Farm, a farm owned by the Maine Agricultural and Forest Experiment Station. Prior to use in this experiment the soil was sieved to remove any small animals (e.g., nematodes and mites) that might interfere. This soil was chosen due to its availability and for its mineral quality, which is typical of lawn and garden soil where the fungal biocontrol would be used in natural settings. The pH of the soil was altered for the experimental treatments using highly acidic and basic additions of 1M hydrochloric acid and 0.1M sodium hydroxide, respectively. The pH of the original soil was 6.0 and the target pH of each soil treatment was 5.0 and 7.0 as they represent the range of Maine soil where this fungal biocontrol would be used (Rourke and Beek 1968). Due to the limitations of time, soils were only able to be altered to have a pH of 5.4 and 6.5, as measured with a benchtop pH meter. Although neither met the target pH, the spread between the two was deemed large enough to allow for differences in results.

Fungal Biocontrol

The species of fungus used in this experiment was *Beauveria bassiana*. It was sold as BotaniGard® 22WP in the form of fungal conidia (spores) and purchased from Arbico Organics. The strain of this specific fungus is the GHA strain with an EPA registration number of 82074-2. Application amount and frequency was determined by the manufacturer's directions. The recommended application concentration for high density pests was used, which was 2 pounds of fungal spore per 100 gallons of water over 20,000 square feet. Due to a calculation error, the actual concentration applied was about 10% higher at 2.2 pounds per 100 gallons. This yields a concentration of 6×10^9 conidia per milliliter. The method of application was spraying the fungal suspension with a household spray bottle onto the soil of each container unit. A total of 5 ml of suspension was applied to each container unit, equating to about 65mg of product, or 3×10^{10} conidia per unit. After the initial application to the surface of the soil and mixing, the fungal biocontrol was applied every seven days as per the product instructions without agitating the soil. On subsequent applications, ticks remained in the container units and were exposed directly to the fungal spores.

Statistical Methods

Microsoft Excel was used for statistical analysis and data visualization. Two-tailed Student's t-tests were used for statistical analysis. Significant differences were declared between groups that had a P value less than 0.05.

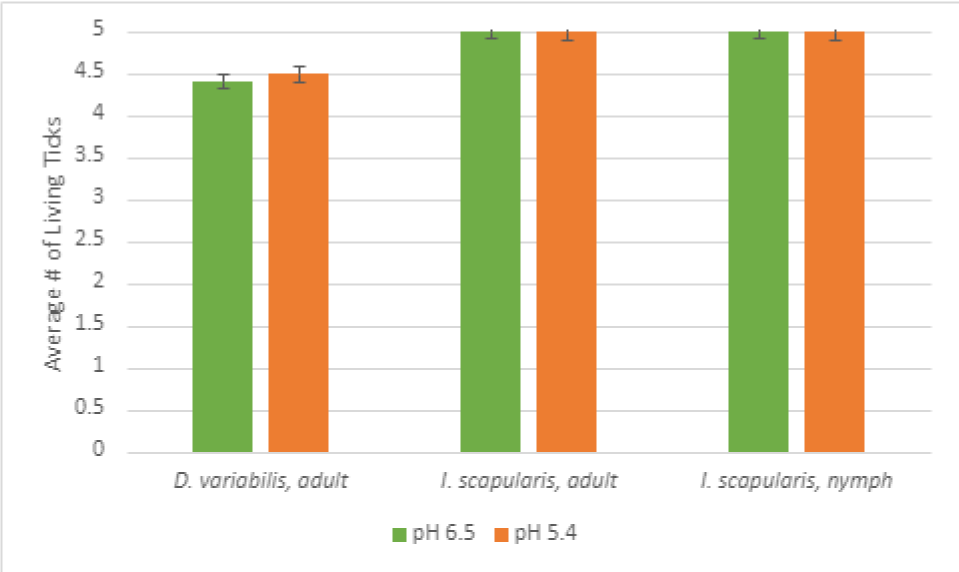

Figure 4. Effect of soil pH on the number of surviving ticks at day 28 across species and life stage.

No significant change in tick survival was found across different soil pH for any tick species or life stage [*D. variabilis* (P = 0.88), *I. scapularis* adults and *I. scapularis* nymphs (no difference), Figure 4]. The only overall tick mortality observed in these two soil treatments was with *D. variabilis*.

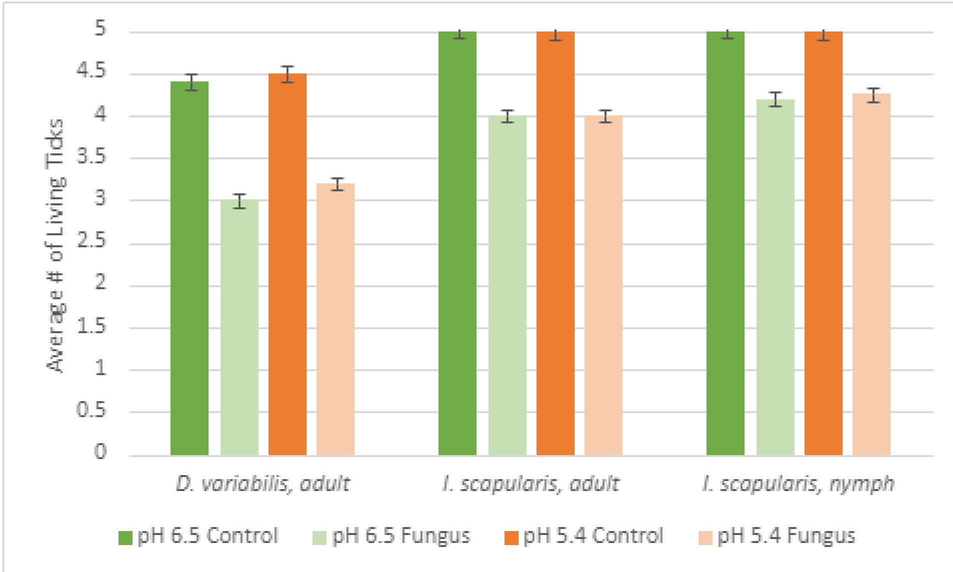

Figure 5. Effects of soil pH and fungal biocontrol on number of ticks surviving at day 28 across tick species and life stage.

All tick and soil treatments saw a significant increase in tick mortality with the fungal application compared to the control [*D. variabilis* (P = 0.01), *I. scapularis* adults (P = 0.01), *I. scapularis* nymphs (P < 0.01), Figure 5], ranging from a 0.75 tick increase to a 1.4 tick increase in average tick mortality in *I. scapularis* nymphs and *D. variabilis* adults, respectively. Tick mortality was significantly higher in soil pH 6.5 fungal treatments in *D. variabilis* adults than in *I. scapularis* nymphs (P = 0.03), with 68% of *D. variabilis* ticks and 80% of *I. scapularis* nymphs surviving as measured against respective controls. No similar significant difference was found between *D. variabilis* adults and *I. scapularis* adults (P = 0.09) or *I. scapularis* adults and nymphs (P = 0.61). No significant difference was found between mortality between tick types in the fungal treatment for soil pH 5.4 [*D. variabilis* adults to *I. scapularis* adults (P = 0.14), *D. variabilis* adults to *I. scapularis* nymphs (P = 0.06), *I. scapularis* adults to *I. scapularis* nymphs (P = 0.57)].

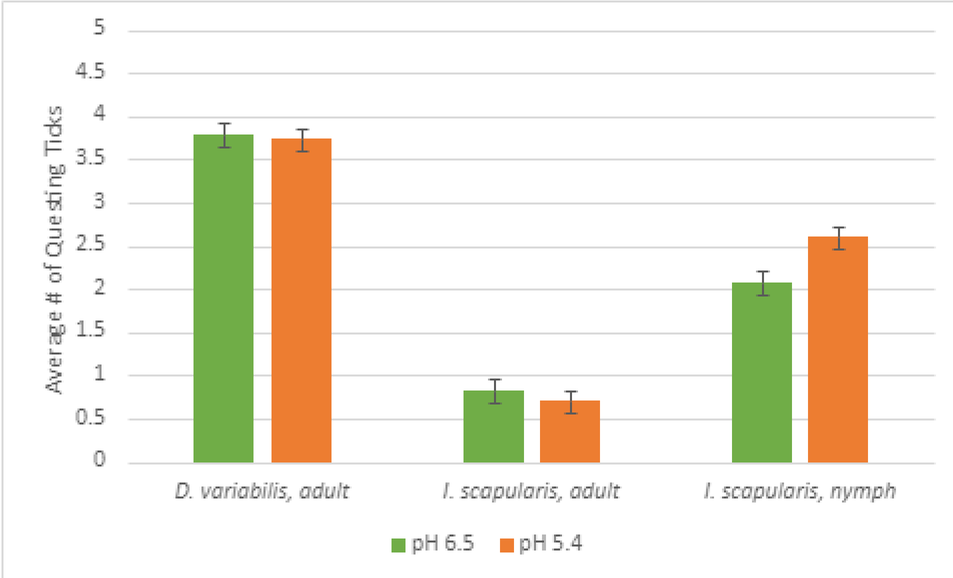

Figure 6. Effect of soil pH on the number of ticks questing across species and life stages.

Little significant difference was observed in tick questing within species and life stages across soil pH treatments. *Ixodes scapularis* nymphs were found to quest more in soil pH 5.4 compared to pH 6.5 ($P < 0.01$, Figure 6), on average by 0.5 ticks. No significant difference between soil pH treatments was found in questing behavior for *D. variabilis* adults ($P = 0.77$) or *I. scapularis* adults ($P = 0.32$).

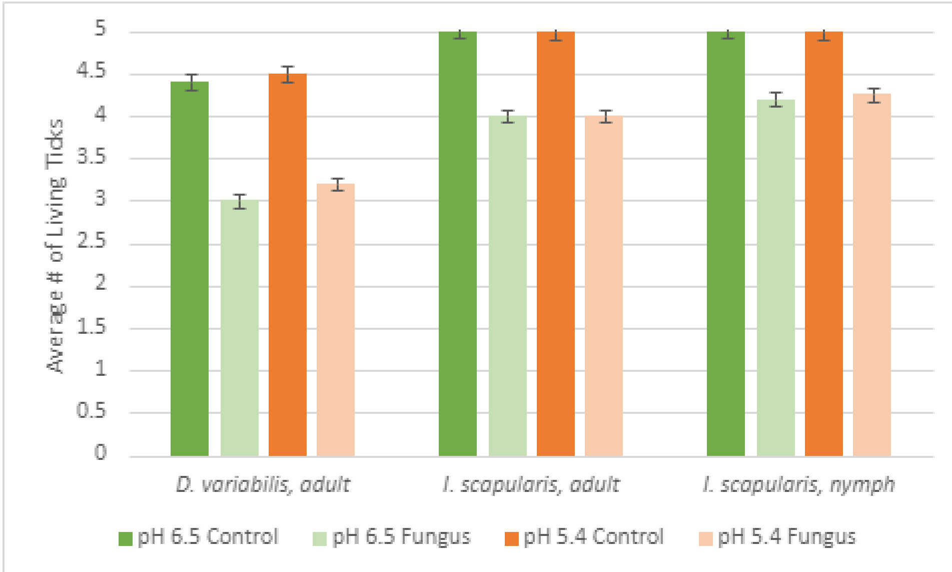

Figure 7. Effects of soil pH and fungal biocontrol on number of ticks questing across tick species and life stage.

A significant increase in tick questing was observed across all tick species and life stages in soil pH 6.5. In the case of *I. scapularis* adults, the increase was double ($P < 0.01$, Figure 7), with an average of 0.8 ticks questing in the control and 1.6 ticks questing with the fungal application. *Dermacentor variabilis* adults ($P < 0.01$) and *I. scapularis* ($P < 0.01$) nymphs also had significant increases in tick questing in the fungal application treatment when compared to controls, with increases of 0.5 ticks and 0.7 ticks, respectively. The questing results from soil pH 5.4 were more varied across tick types. A slight but insignificant decrease in questing with the fungal biocontrol was seen in *D. variabilis* adults ($P = 0.39$). In *I. scapularis* adults, a significant decrease was observed ($P = 0.03$) with the fungal application. Similar to the soil pH 6.5 treatment, the fungal application increased questing in *I. scapularis* but without statistical significance ($P = 0.13$).

DISCUSSION

The effects of *Beauveria bassiana* on tick survival and its efficacy as a tick control is reaffirmed in this experiment as with past research (Kaaya and Hassan 2000). *Beauveria bassiana* was found to increase tick mortality, with similar mortality rates seen in previous investigations (Kirkland et al. 2004). Another study (Fischhoff et al. 2018) found no significant impact in questing behavior from fungal biocontrol treatments, which contradicts these findings of significant changes in questing behavior in various tick species and life stages. However, this previous study used a different fungal biocontrol, *Metarhizium anisopliae*, that may have different impacts on questing behavior than the species used in this study, *B. bassiana*.

At the start of this experiment, I hypothesized that soil pH would have an effect on tick mortality or behavior, and this was found to be mostly false across tick types. Different soil pH did not impact tick mortality, which indicates that soil pH is not an important factor in tick survival within the range used (pH 5.4 to 6.5). Previous field studies have found some connections between more acidic soils and lower tick presence (Guerra et al. 2002), while others have found no associations between the two (Milne 1944). These studies only collected ticks and looked for associations between tick presence and soil characteristics. Guerra et al. (2002) suggests that soil acidity is conducive to coniferous forest growth and suggested the tree type to be the influential factor. This may be the case as we did not observe lower soil pH significantly affecting tick survival. The impact of soil pH on tick questing behavior has not been previously investigated but has been looked at with different behaviors in other arthropods. These studies found variable effects of soil pH on behavior, depending on the species

(Sastrodihardjo and Straalen 1993; Zimmer et al. 2000). However, both studies only looked at behavior as the movement towards preferred soil pH in some woodlice (Order: Isopoda) and millipedes (Class: Diplopoda), and no specific behaviors beyond this were pursued. The results from our research suggest that *I. scapularis* nymphs quest more on more acidic soils. Nymphs do not have a fully hardened cuticle (Kocan et al. 2015) and are more susceptible to unfavorable environmental conditions, such as temperature and humidity, due to their smaller body weight (Chilton and Bull 1993). There may be a behavioral response to the potentially harmful effects that the more acidic soil has on the nymphs. Although it was recorded as questing in this experiment, this behavior may have been one of avoidance where the nymphs reduce contact with the potentially harmful soil. Considering soil pH had no observed effect on tick mortality, this may be due to the increased nymph questing on acidic soils, reducing the time exposed to the soil. Future research would be warranted to better understand this relationship. Although soils are complex systems, this finding indicates that people active in areas with more acidic soil are at greater risk of coming into contact with *I. scapularis* nymphs, which are the most clinically significant type (Gray et al. 2002). The lack of increased questing behavior in *D. variabilis* adults and *I. scapularis* adults, as well as no effect on tick mortality in differing soil pH, suggests soil pH between 5.4 and 6.5 should not be considered an independent factor in determining tick habitat ranges.

The primary reason for this research was to investigate if soil pH affected the efficacy of *B. bassiana* as a tick control. Our results show that this biocontrol was effective at reducing tick survival and that its efficacy was not affected by the values of soil pH used (pH 5.4 to 6.5). Research has shown *B. bassiana* prefers inhabiting more neutral pH soils in nature (Quesada-Moraga et al. 2007), however, this pattern was not found in its efficacy as a tick biocontrol. This makes sense as the mechanism of entomopathogenic fungi is through contact and attachment between fungal spores and insects (St. Leger et al. 1986; More et al. 2017). Applications of fungal biocontrols typically use high concentrations of spores and frequent application to maximize the amount of spore coming into contact with the pests, increasing its efficacy (Ortiz-Urquiza and Keyhani 2013). This finding confirms that *B. bassiana* efficacy as a biocontrol is not affected by the soil pH values studied and can be used similarly in neutral to slightly acidic soils.

The questing behavior of these ticks were assessed under conditions of *B. bassiana* application. I had hypothesized that questing behavior would be reduced when *B. bassiana* was applied due to the initial symptoms of infection, but the results were rather mixed. In the soil treatment with pH 6.5, the questing behavior of all tick types significantly increased. For the more acidic soil, the only significant change was a decrease in questing behavior for *I. scapularis* adults. Manoussopoulos et al. (2019) and Roy et al. (2006) both demonstrated the potential of entomopathogenic fungi to cause behavioral change in other arthropods. Manoussopoulos et al. (2019) looked at an agriculturally damaging aphid, *Myzus persicae*, and recorded specific behaviors that were altered or omitted in individuals exposed to *B. bassiana*. It may be that a similar process is undergone when ticks are exposed to *B. bassiana*. Behavioral defenses against entomopathogenic fungal exposure have been observed in a wide range of arthropods. Examples include mutual grooming in termite species (Yanagawa et al. 2008) and sunbathing to reduce spore germination in the western boxelder bug (Schwarz et al. 2012;

Fernandez et al. 2007). Perhaps ticks possess a similar adaptation to recognize fungal presence in soil and avoid contact with such soil. Further research will need to be conducted to determine if these conjectures are valid.

The fungus did not have a similar impact on tick behavior in the more acidic soil, suggesting that although the fungus's primary mechanism of infection is through contact, the soil conditions may still play a role. As shown in previous research, *B. bassiana* prefers inhabiting more neutral soils in nature (Quesada-Moraga et al. 2007; Groden and Lockwood 1991). It could be that the behavioral effects of *B. bassiana* on ticks require the establishment of the fungus in the soil, where the more acidic soil is not as suitable for the fungus's growth, leading to mixed results in the more acidic soil. Since this relationship is not understood and the results of the questing behavior in soil pH 5.4 are mixed, further investigation would be warranted. Despite this, the overall increase in questing behavior, particularly in neutral pH soil, raises the question of how efficacious *B. bassiana* is as a tick control. Although it increases tick mortality, it also increases tick questing behavior which increases the likelihood of a tick finding a human host. Future work should focus on this interplay between fungus and behavior in the field to better understand this relationship with more varied conditions.

The results of this study, though valuable, were not achieved in a perfect set up or manner of data collection. As previously discussed, entomopathogenic fungi have been isolated from soils across the world, so it's possible some were present in the soil used in this experiment. Autoclaving the soil before use would have removed such variables, as well as other microbial life that was not sieved out. However, these microorganisms can be vital to soil microhabitats, further reducing the similarities to field conditions (Carter et al. 2007). Even though entomopathogenic fungi do not infect hosts at high rates in nature (Roy and Pell 2000), this is a confounding variable that future, similar research may want to remove. The soil moisture was kept relatively similar among all individual container units, but some difference was inevitable. Soils with a higher moisture content may aid the growth of entomopathogenic fungi, increasing its virulence and probability of infection (Garrido-Jurado 2011, Zhioua et al. 1999), thus it would be better to standardize the method of maintaining equal soil moisture content.

Ixodes scapularis and *D. variabilis* and the diseases they transmit burden our public healthcare system and cost billions in economic losses each year, and effective methods to control them are becoming increasingly sought after. In this study we have examined the effects of various soil pH levels on a useful form of tick control, *B. bassiana*, as well as the behavioral effects of this fungus on ticks. The finding that soil pH alone does not affect tick survival and has a slight impact on questing behavior for the values studied can help us focus on other abiotic factors. We found *B. bassiana* had no change in mortality across the different soil pH levels, which will be helpful in using this fungal biocontrol in a broad range of soils. However, we also found that *B. bassiana* increased the questing behavior of ticks on neutral pH soil and had mixed results in more acidic soil. This potentially limits the usefulness of *B. bassiana* as an effective tick control. Future research should investigate this connection between fungus and questing behavior, specifically, and preferably in the field to provide a better understanding of this relationship in a complex environment.

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