SUSCEPTIBILITY OF WINTER TICK LARVAE AND EGGS TO ENTOMOPATHOGENIC FUNGI - *BEAUVERIA BASSIANA*, *BEAUVERIA CALEDONICA*, *METARHIZIUM ANISOPLIAE*, AND *SCOPULARIOPSIS BREVICAULIS*

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ABSTRACT: An isolate of the soil fungus *Scopulariopsis brevicaulis* was identified from the surface of female winter ticks (*Dermacentor albipictus*) collected from recently dead moose (*Alces alces*) calves in New Hampshire in the northeastern United States. It was the sole isolate, and it matched with 98% nucITS similarity (molecular systematics Blast match) to *S. brevicaulis* species from soil and other tick species. Inoculation of tick larvae and eggs with 10^8 spores/mL + 0.05% Tween (aqueous inoculum) resulted in mortality, reduced survival time, and recovery of *S. brevicaulis* from within tick tissues. Rapid water loss and death from dehydration were the pathogenic consequences of the fungal infection. Three entomopathogenic fungal isolates from laboratory culture (*Beauveria bassiana*, *B. caledonica*, and *Metarhizium anisopliae*) inoculated concurrently at the same dose, were slightly less pathogenic to eggs than larvae of winter ticks. We conclude that *S. brevicaulis* imposes a limitation on the free-living stages of the winter tick population in specific environmental conditions, but commercial fungal treatments as used in local situations to control ticks, are impractical as a means of controlling winter tick density across moose habitats.

ALCES VOL. 53: 41–51 (2017)

Key words: *Alces alces*, *Dermacentor albipictus*, moose, pathogenic fungi, *Scopulariopsis brevicaulis*, survival, water balance, winter ticks

INTRODUCTION

Winter ticks (*Dermacentor albipictus*) periodically cause high calf mortality in moose (*Alces alces*) populations in the northeastern United States and southern Canada due to numerous interactive stressors, including extreme blood loss, problems with thermoregulation, and incidence of pathogenic bacteria (McLaughlin and Addison 1986, Campbell et al. 1994, Samuel 2004, Musante et al. 2007, Addison and McLaughlin 2014, Jones 2016). As a one-host tick, it feeds, molts, and mates on the same individual moose from approximately late September to mid-April. Once a female has mated and fully engorged, she drops to and crawls on the ground to lay eggs in a suitable, moisture-rich reprieve in soil and leaf litter. Eggs hatch in about a month and the larvae enter a resting period during summer, regaining activity and questing for a host during autumn (Drew and Samuel 1985, Addison and McLaughlin 1988). The moisture-rich microhabitats in the northeastern forest preferred for oviposition, hatching, and quiescence (Yoder et al. 2016) expose adult females, eggs, and unfed larvae (the sole transmission stage) to numerous filamentous soil fungi (Tuininga et al. 2009) that

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are principally saprobes and agents of decay, but could be infective to ticks.

Fungal infection is a primary source of mortality in ticks and is often the basis for their biological control as with the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, and *Scopulariopsis brevicaulis* (Kirkland et al. 2004, Suleiman et al. 2013). These fungi are regular soil saprobes that produce copious amounts of spores (Barnett and Hunter 2003), live free in soil, and can invade a perfectly healthy tick. Presumably, ticks come into contact and are infected by way of spores that adhere to the cuticular surface, germinate, and then colonize via hyphae that gain internal access via the mouth, anus, genital pore, cuticular gland openings, and between leg segments. Infection often results from a single fungal species that exploits the tick (Yoder et al. 2008), such that an infected tick is essentially a pure culture of the infectious agent. Once the infection proliferates, the fungal hyphae typically protrude from the cuticular glands around the body. After death, the tick dries out, the body flattens, and fungal hyphae typically protrude from the mouth spreading over the front pair of legs, eventually enveloping the carcass within the fungal mycelium. An increased rate of water loss serves as an indicator of establishment and progression of the infection (Cradock and Needham 2011). Not all entomopathogenic fungi are universally pathogenic to all ticks; for example, *B. bassiana* and *M. anisopliae* are particularly ineffective against the American dog tick (*Dermacentor variabilis*; Kirkland et al. 2004), a close relative of the winter tick.

We suspected that a fungal mortality agent existed for winter ticks during routine handling of specimens used in related studies with engorged females collected from dead moose in New Hampshire (Yoder et al. 2016). Under relatively moist and warm conditions (93% RH, 25 °C), some of the engorged females died and became moldy during storage. Further, we observed healthy ticks die and became covered with a whitish mold when housed in the same storage containers with moldy ticks. The objectives of this study were to 1) isolate and determine the fungus that infected the engorged female ticks, 2) determine if the fungus was pathogenic to winter tick eggs and larvae (ground-dwelling stages), and 3) compare the relative pathogenicity of the isolated fungus with the entomopathogenic fungi *B. bassiana* and *M. anisopliae* in the context of biological control agents.

METHODS

Study area

Winter ticks were collected from dead moose in eastern Coos County, New Hampshire, an area considered the best habitat and of highest moose density in New Hampshire (see Jones 2016). The area is dominated by mountainous terrain (elevation 300 to 1200 m) bordered by lowland valleys containing a myriad of lakes, ponds, and river systems. The dominant cover type is northern hardwood forest with a mix of American beech (*Fagus grandifolia*), yellow birch (*Betula alleghaniensis*), and sugar maple (*Acer saccharum*); balsam fir (*Albies balsamea*), red spruce (*Picea rubens*), and white pine (*Pinus strobus*) occur on more poorly drained sites. Monthly precipitation, mean ambient temperature, precipitation, snow depth, and other weather variables were available from the National Climatic Data Center (44827 'N, 71811 'W) weather station in Berlin, New Hampshire (#270690/99999) located centrally in the study area at 283 m elevation. Annual ambient temperature ranged from 30 to −30 °C, annual precipitation from 91 to 123 cm, and maximum snow depth from 50 to 70 cm.

Tick collection

Aseptic technique was followed in the laboratory using materials that were autoclave-sterilized (121 °C, 19 psi, 15 min),

flamed off using a Bunsen burner, treated with 95% ethanol, or purchased sterile from the manufacturer. Sterile, powder-free gloves were worn when necessary (Microlex Co., Reno, Nevada, USA).

Ticks were collected from 5, 10.5 month-old moose that had been captured and radio-collared in January 2015; death occurred 24-36 h prior to collection. All moose were emaciated and mortality was attributed to anemia and hypoproteinemia from excessive blood loss associated with >30,000 winter ticks/animal (Jones 2016). Ticks were collected from the neck, shoulder, abdomen, and rump of moose, and included nymphs and adults in various stages of feeding and unfed specimens. All ticks were identified as *Dermacentor albipictus* from keys (Brinton et al. 1965). Fed females were placed individually into 50 mL polypropylene centrifuge tubes (Fisher Scientific, Pittsburgh, Pennsylvania, USA) within Whirl-Pak bags (Nasco, Salida, California, USA) and transported to the laboratory in 5 L coolers kept at ~15 °C with cold packs (Koolit; FDC Packaging, Medfield, Massachusetts, USA).

In the laboratory, each fed female was transferred to a fresh tube and stored at 93% RH (SD ± 0.5% RH; Winston and Bates 1960) in 3000 mL glass desiccators at 25 ± 0.5 °C, 10L:14D (programmable incubator; Fisher). Subsequent hatched larvae were identified as *D. albipictus* using keys (20 slide-mounted larvae); no other species of larvae was identified. At the time of collection, all fed females were in healthy condition in that their body was plump and blood-filled, and they could crawl 5 body lengths.

Isolation and identification of fungi from tick cadavers

The methods used to isolate fungi from moldy ticks, the preparation and spore concentration of inoculum, use of Tween as an emulsifier (dispersing agent), treatment of larvae and eggs, and reisolation of fungi were modified from previous studies with ticks and entomopathogenic fungi (Fernandes et al. 2004, Kirkland et al. 2004, Tuininga et al. 2009, Suleiman et al. 2013). Moldy engorged females were used to culture fungi from pieces of hyphae scraped from their carcasses. Individual hyphae were plated individually onto solidified potato dextrose agar (PDA; Fisher) in disposable 100 × 15 mm Petri plates (Fisher) that were incubated in darkness at 25 °C. The fungus was purified with 3 rounds of subculturing hyphal tips, each utilizing the advancing edge of a 3-4 week old mycelium. Pure cultures were identified at the University of Alberta Microfungus Collection and Herbarium (UAMH) Centre for Global Microfungal Biodiversity at the Gage Research Institute (Toronto, Ontario, Canada). The gene sequenced for identification was nucITS (internal transcribed spacer region) and primers ITS5/ITS4 were used for amplification. ClustalX software in MEGA5 was used for aligning nucITS sequences (Gen Bank) to compare with other species at the Department of Biological Sciences, University of Cincinnati (Cincinnati, Ohio, USA).

Preparation of fungal inoculum

An aqueous inoculum was prepared from 1 month-old sporing PDA cultures in phosphate buffered saline (PBS, pH 7.5) + 0.05% Tween 20 (Fisher). Spores were scraped from the plates into PBS, purified, and the concentration was adjusted to 1.4×10^8 spores/mL with a 0.1% dye exclusion (AO Spencer Bright-Line Hemocytometer, St. Louis, Missouri, USA). Identical preparation of aqueous inocula (each at 1.4×10^8 spores/mL in PBS + Tween) was performed for *Beauveria bassiana*, *B. caledonica*, and *Metarhizium anisopliae* from the Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (ARSEF) associated with the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) (Ithaca, New York, USA) (see Table 1 for isolate number information).

Table 1. Entomopathogenic fungi used in this study. The *Scopulariopsis brevicaulis* isolate 11903 is the new isolate collected from dead winter ticks originating from New Hampshire, USA.

Fungus and isolate#	Host	Origin	Deposited
Beauveria bassiana 149	*Leptinotarsa decemlineata* (Colorado potato beetle)	France, Europe	ARSEF[1]
Beauveria caledonica 11821	*Hadenoecus cumberlandicus* (Cave cricket)	Kentucky, USA	UAMH[2]
Metarhizium anisopliae 23	*Conoderus* sp. (Click beetle)	North Carolina, USA	ARSEF
Scopulariopsis brevicaulis 11903	*Dermacentor albipictus* (Winter tick)	New Hampshire, USA	UAMH

[1]ARSEF, USDA-ARS Collection of Entomopathogenic Fungal Cultures, Ithaca, New York, USA.
[2] UAMH, UAMH Centre for Global Microfungal Biodiversity, Toronto, Ontario, Canada.

We used these entomopathogenic fungi as positive controls; PBS + 0.05% Tween served as the negative control.

Treatment with inoculum

Ten larvae were placed into a 1.5 mL microcentrifuge tube (Fisher) containing 1 mL of inoculum that was gently agitated for 2 min, and then poured onto filter paper (No. 3, Whatman, Hillsboro, Oregon, USA). Actively crawling larvae were collected and placed into a clean 1.5 mL microcentrifuge tube; a hole was punched through the tube lid and covered with mesh. It was placed at 80% RH (Winston and Bates 1960), 25 °C, and 10h:14h L:D cycle in a sealed glass desiccator. Dead larvae were identified with a 40× light microscope; i.e., larvae with curled legs, deflated opisthosoma, and no movement. The identical inoculum treatment was used with eggs and death was assumed when the eggshell chorion showed sign of collapse; eggs in such condition fail to hatch based on water balance studies (Yoder et al. 2016). The experiment was complete after all larvae died, including those in the PBS + Tween control, and after egg hatching occurred.

Reisolation of fungi

In accordance with Koch's postulates as confirmation of pathogenicity, dead larvae and eggs were prepared for reisolation by fungus culturing. Briefly, each was surface sterilized twice for 1 min in a mild bleach solution (18:1:1 ratio of deionoized water: absolute ethanol:5.25% NaOCl by volume), with a final rinse in water. It was then halved by scalpel and the portions were embedded in PDA, each in its own plate, and incubated in darkness at 25 °C. Tips of hyphae that could be traced as originating from the internal body contents (40/45× microscopy) were removed as a 1 cm^3 block for sub-culturing and identification. The fungus was identified with standard keys (Barnett and Hunter 2003) and pure culture comparison to the original fungus isolates used to prepare the inoculum. Defining characteristics were identified by using colony obverse and reverse, and spore size and shape under oil (1000×).

Larvae were 4-6 weeks old and eggs were 2–3 weeks post-oviposition; all were healthy at the time of treatment. Eggs were full and rounded, with a visible accumulation of white guanine through the eggshell that is a developmental landmark of regular embryonic development. Larvae crawled about actively, could self-right, and crawl 5 body lengths.

Data are the mean ± SE of 10 replicates from 10 specimens each (n = 100). An analysis of covariance was used to test data

(ANCOVA; $P = 0.05$; SPSS 14.0, Microsoft Excel and Minitab, Chicago, Illinois, USA). Survival times were compared with the *t* statistic utilizing a Kaplan-Meier survival curve with a log rank test. An Abbott correction for mortality data and logit-transformation for percentage data were used prior to analysis.

Water balance experiments

Eggs and larvae were analyzed similarly. After treatment with inoculum (4 d post-treatment), each specimen was weighed individually with a microbalance (SD ± 0.2 µg precision, ± 6 µg accuracy at 1 mg; Cahn Ventron Co., Cerritos, California, USA). This measurement was made in <1 min without enclosures or anesthesia. Standard kinetic model equations were used to determine water balance characteristics based on the mass measurements (Yoder et al. 2016). All specimens were predesiccated by 4-6% so that the change in mass reflected the change in body water content. The percent water content was determined by weighing the specimen (initial, fresh mass, f), drying it to constant mass (dry mass, d) at 90 °C in a drying oven (< ± 2 °C; Blue M Electric Co., Chicago, Illinois, USA), and calculating the difference between these measurements: 100% $(f - d)/f$, where $f - d$ is the water mass, m.

The dehydration tolerance limit was determined by weighing the specimen, placing the specimen at 33% RH (Winston and Bates 1960) in a glass desiccator at 25 °C, and then reweighing the specimen at time intervals. The critical mass measurement for larvae was the point at which a larva was unable to self-right and crawl 5 body lengths, and for eggs when the eggshell chorion began to collapse. Specimens at their critical mass were transferred to a 90 °C drying oven to determine dry mass (d). The difference between critical mass and dry mass was defined as the critical water mass (m_c). The amount of water loss that was sustained between the initial water mass (m_0) and m_c was defined as the dehydration tolerance limit expressed as a percentage: 100% $(m_c - m_0)/m_0$.

The water loss rate (respiratory + integumental water loss) was measured at 0% RH because this is the only condition where the rate is exponential, allowing it to be derived from the slope of a regression line. The 0% RH condition was maintained with anhydrous calcium sulfate at 25 °C in a glass desiccator (Drierite; 1.5 x 10^{-2}% RH; W. A. Hammond Drierite Company, Xenia, Ohio, USA). The specimen was weighed, placed at 0% RH, and reweighed 5 times at various intervals. The specimen was then transferred to a 90 °C drying oven to achieve dry mass (d) and water mass (m) was calculated by subtraction. The water loss rate ($-kt$) was determined from $m_t = m_0 \exp\text{-}kt$, where m_t is the water mass at any time t, and m_0 is the initial water mass.

Each water balance characteristic was based on a sample size of 100; 10 replicates of 10 specimens each. Data (the mean ± SE) were tested using ANCOVA ($P = 0.05$). Percentage data were logit-transformed prior to analysis, and regression lines were compared with a test to compare characteristics and slopes from multiple regression lines (SPSS 14.0, Microsoft Excel and Minitab).

RESULTS

Identification of fungus (*S. brevicaulis*)

The fungus that was scraped and isolated from a moldy, fed female was identified as *Scopulariopsis brevicaulis* (Sacc.) Bainier (UAMH isolate 11903); this isolate originated from a single tick. The identification was based on sequence analysis and 99% similarity of the ITS region with other *S. brevicaulis* strains and the basic morphological characters of the group. The *S. brevicaulis* isolate 11903 is deposited at The UAMC Centre in Toronto, Ontario, Canada (Table 1). *Scopulariopsis brevicaulis* was the most common isolate and was recovered in pure culture from 21 moldy females (identification based

on pure culture comparison to authentic *S. brevicaulis* strain 11903). Not all of the collected fed female ticks from moose had a fungal infection (n = 21 of >110), not all fed female ticks were collected from the same moose, and not all fed female ticks were collected from moose during the same week. The inoculums for testing were made from a single isolate culture (i.e., not batched from all 21 positive ticks). Other fungi recovered less frequently (<15%) from fed female ticks included *Aspergillus* spp., *Penicillium* spp., and *Paecilomyces* spp.

Effect of *S. brevicaulis* 11903 on survival on larval ticks and eggs

Survival was reduced in *S. brevicaulis*-treated larvae ($P < 0.05$; Fig. 1) that lasted 14.0 d (7.5 d for 50% of larvae) versus 18.0 d for control larvae (11.2 d for 50% of larvae). The recovery of *S. brevicaulis* from dead larvae at the end of the experiment was 90.4 ± 2.2% in the treated group and 6.3 ± 2.9% in the control group ($P < 0.05$). Similarly, treated eggs had lower survival and hatching rate, and higher recovery of *S. brevicaulis* from unhatched eggs than the control group (Table 2).

Effect of *S. brevicaulis* 11903 on water loss on larval ticks and eggs

Scopulariopsis brevicaulis-treated larvae lost water >2 × faster ($P < 0.05$) than control larvae (4.39 ± 0.07%/h vs. 1.84 ± 0.04%/h; Fig. 2). The recovery of *S. brevicaulis* was ~5× higher ($P < 0.05$) in the treated (86.7 ± 4.7%) than control group (17.8 ± 3.9%). *Scopulariopsis brevicaulis* was recovered internally from dead larvae in the treatment group, whereas only dead larvae tested positive for *S. brevicaulis* in the control group ($P < 0.05$). Similarly, eggs had higher ($P < 0.05$) water loss rate in the treatment (1.12 ± 0.019%/h) than control group (0.71 ± 0.033%/h) (Fig. 2). Dead eggs also had higher ($P < 0.05$) internal recovery of *S. brevicaulis* in the treated (71.1 ± 2.9%) than control group (4.4 ± 3.9%). There was greater recovery of *S. brevicaulis* in larvae than eggs ($P < 0.05$).

After 4 days post-treatment, the fresh mass, water mass, and % water content were similar for eggs and larvae in the *S. brevicaulis*-treated and control groups (Table 3), which reflected the similarity of the water:dry mass

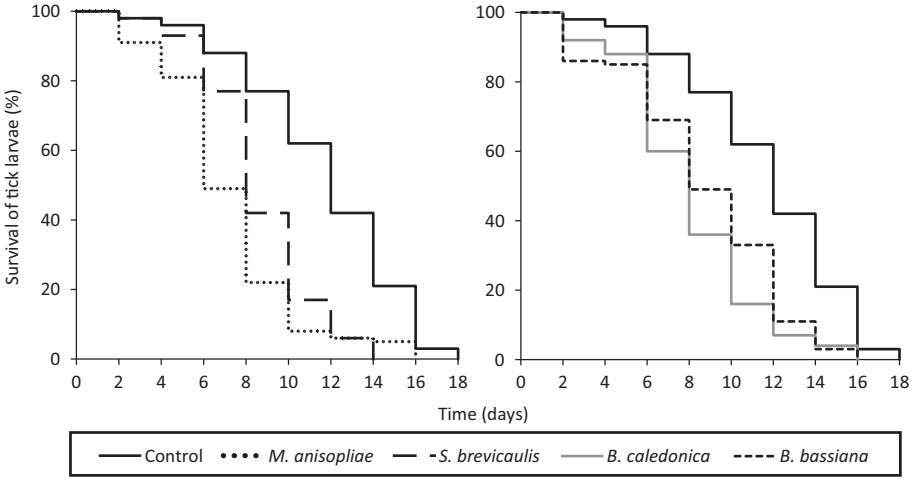

Fig. 1. Survivorship curves for unfed larvae of *Dermacentor albipictus* after treatment with *Metarhizium anisopliae*, *Scopulariopsis brevicaulis*, *Beauveria caledonica*, or *B. bassiana* (in order from left to right on the graph). The control on both plots is the solid black line for comparison with the treatments. Each point is the mean of 100 larvae (± SE ≤ 2.1).

Table 2. Mortality characteristics associated with *Beauveria bassiana*, *B. caledonica*, *Metarhizium anisopliae*, or *Scopulariopsis brevicaulis* to eggs of *Dermacentor albipictus*. Values (the mean ± SE; n = 100 eggs) followed by the same superscript letter within a column are not significantly different ($P < 0.05$). Eggs in the control had an incubation time of 44.6 ± 2.1 days.

	Days elapsed before		
Treatment	chorion collapsed (50% of eggs)	%/100 eggs that hatched	% dead eggs positive for test fungus
Control (PBS + Tween)	Not observed (0.0)[a]	82.6 ± 5.5[a]	11.8 ± 2.0[a]
Test (10^8 spores/mL)			
B. bassiana	16.1 ± 3.1[b]	43.7 ± 5.2[b]	73.2 ± 2.0[b]
B. caledonica	13.2 ± 1.7[b]	46.2 ± 3.0[b]	62.9 ± 1.4[c]
M. anisopliae	11.0 ± 2.2[c]	44.1 ± 4.7[b]	80.4 ± 3.2[d]
S. brevicaulis	8.4 ± 2.1[d]	36.6 ± 4.3[c]	76.2 ± 2.8[b]

ratios (m/d): 1.44 and 1.28 for larvae and 1.81 and 1.96 for eggs in the *S. brevicaulis*-treated and control groups, respectively. In all cases, water mass was a positive correlate of dry mass in larvae ($R \geq 0.90$ for control and ≥ 0.94 for *S. brevicaulis*-treated) and eggs ($R \geq 0.89$ for control and ≥ 0.91 for *S. brevicaulis*-treated) ($P < 0.001$).

Comparative observations with other entomopathogenic fungi on larval ticks and eggs

Larval survival was reduced ($P < 0.05$) by treatment with *B. bassiana*, *B. caledonica*, and *M. anisopliae*: 8.1, 7.0, and 8.3 d, respectively, compared to 11.2 days for 50% of control larvae (Fig. 1). At the end of the

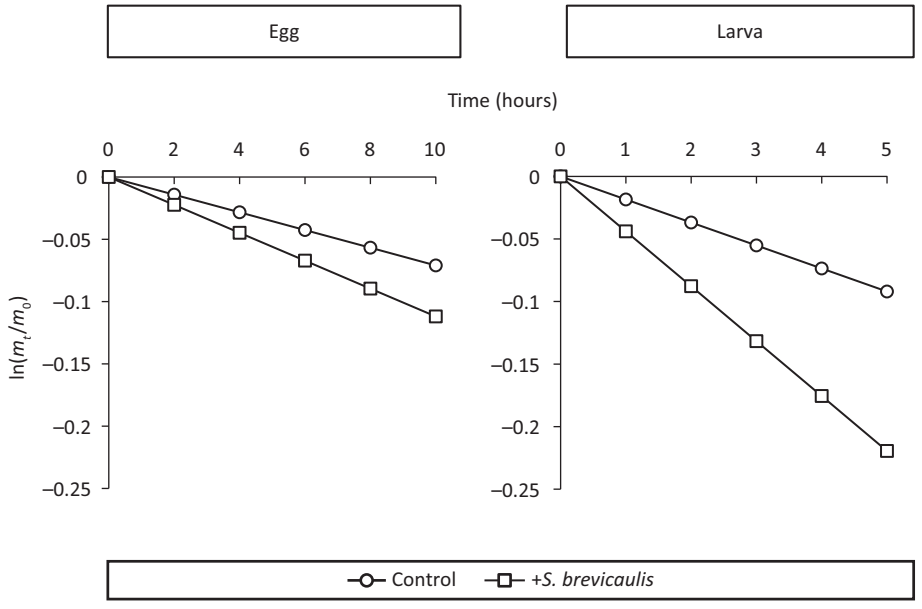

Fig. 2. Water loss rate of unfed larvae and eggs of *Dermacentor albipictus* after treatment with *Scopulariopsis brevicaulis*. The water loss rate is derived from the slope of the regression line; m_t = water mass at time t; m_0 = initial water mass. Each point is the mean of 100 specimens.

Table 3. Water content and dehydration tolerance of unfed larvae and eggs of *Dermacentor albipictus* that were treated with *Scopulariopsis brevicaulis*. Values (the mean ± SE) followed by the same superscript letter within a column are not significantly different ($P < 0.05$); n = 100 specimens each.

Stage	Fresh mass (mg)	Water mass (mg)	Water content (%)	Dehydration tolerance (%)
Larva				
Control	0.041 ± 0.008[a]	0.023 ± 0.006[a]	56.10 ± 1.43[a]	21.73 ± 0.62[a]
+ *S. brevicaulis*	0.044 ± 0.010[a]	0.026 ± 0.008[a]	59.09 ± 1.28[a]	24.06 ± 0.49[a]
Egg				
Control	0.073 ± 0.012[b]	0.047 ± 0.005[b]	64.38 ± 1.23[b]	36.19 ± 0.82[b]
+ *S. brevicaulis*	0.068 ± 0.009[b]	0.045 ± 0.008[b]	66.18 ± 1.37[b]	38.44 ± 0.57[b]

experiment, 86.4 ± 2.6% larvae tested positive for *B. bassiana*, 88.6 ± 3.1% tested positive for *B. caledonica*, and 92.9 ± 1.9% tested positive for *M. anisopliae* in their respective treatment groups; none was detected in the control groups.

The 3 fungal treatment groups reduced egg survival and hatching compared to the control group (Table 2). The *S. brevicaulis* treatment had more detrimental effect on hatching than the other fungal treatments. No dead eggs in the control tested positive for *B. bassiana*, *B. caledonica*, or *M. anisopliae*; however, 11.8% were positive for *S. brevicaulis*. The recovery of *B. bassiana*, *B. caledonica*, and *M. anisopliae* was consistently lower ($P < 0.05$) in treated eggs than treated larvae.

DISCUSSION

This study produced 2 novel findings: 1) that larvae and eggs of the winter tick are susceptible to fungal isolates of *B. bassiana, B. caledonica*, and *M. anisopliae*, and 2) that larvae and eggs of the winter tick are susceptible to *S. brevicaulis*, a common soil fungus. Both *B. bassiana* and *M. anisopliae* are approved for tick control in the United States under various commercial formulation trademarks (Stafford and Allan 2010). *Beauveria caledonica* is a pathogen of forest beetles and is used in formulated applications for biological control of bark beetles (*Hylastes ater* and *Hylurgus ligniperda*) in New Zealand (Brownbridge et al. 2010). This is the first instance that an isolate of *B. caledonica* has been tested and shown to be pathogenic against ticks, suggesting promise for biological tick control. It is apparent that *S. brevicaulis* is a pathogen in the study area given its origin from host moose.

Although *B. bassiana*, *M. anisopliae*, *B. caledonica*, and *S. brevicaulis* were pathogenic against both winter tick larvae and eggs, larvae were more vulnerable to infection. It follows that application of an entomopathogenic agent would probably be most effective in the autumn when larvae are active. Similarly, other investigators noted that eggs of other tick species are more resistant to entomopathogenic fungal infection than later life stages, and attribute this to the resistant properties of the eggshell chorion (Fernandes et al. 2004). The entomopathogenic fungi tested were consistent in shared features with other ticks challenged with entomopathogenic fungi in laboratory studies (see Fernandes et al. 2012). Specifically, under warm temperature (25 °C) and moisture levels >80% RH favorable to ticks, there was: 1) confirmation of pathogenicity by Koch's postulates, 2) suitable infection from a topical application, 3) high mortality with a 10^8 spores/mL concentration, and 4) post-treatment infection with 10^8 spores/mL causing death in approximately 10-12 days.

Scopulariopsis brevicaulis is distributed worldwide as a common mold in soil and

forest leaf litter, and is a common saprobe found on fur, hooves, and horns of small and large mammals (Shubina et al. 2013). Thus, seed ticks come into contact with *S. brevicaulis* spores when crawling or as eggs on the ground, or as larvae on host fur. *Scopulariopsis brevicaulis* has also been isolated from moose dung in black spruce (*Picea mariana*) forests in Alberta, Canada (listed as teleomorph *M. brevicaulis* UAMH number 9458; isolator S. P. Abbott), indicating a linkage among *S. brevicaulis*, moose, and moose habitat.

Results here for *S. brevicaulis* show at this inoculum dose (10^8 spores/mL), it kills winter tick. An extended period of wetness can trigger *S. brevicaulis* to proliferate and possibly induce tick mortality. For example, certain engorged specimen females died from *S. brevicaulis* infection after prolonged storage under high moisture (93% RH); effectively, *S. brevicaulis* proliferated and killed the ticks. Exposure to a large inoculum of 10^8 spores/mL of the study set of fungi (*B. bassiana*, *B. caledonica*, *M. anisopliae*, and *S. brevicaulis*) and moisture >80% RH is a lethal combination to eggs, larvae, and adult female winter ticks. The biological significance of *S. brevicaulis* as a limitation on winter tick populations would presumably occur only during optimal environmental conditions (e.g., extended period of high moisture and temperature to enhance spore production), and the likelihood or frequency of such is unknown.

CONCLUSIONS

Due to the potential in mammalian nail and skin infections by *S. brevicaulis* (Lee et al. 2012), precautionary measures should be taken if it is considered as a biological control agent. Biological control of winter ticks is theoretically possible with *B. bassiana* and *M. anisopliae* at 10^8 spores/mL or higher formulations that are consistent with commercially available products. However, commercial applications are typically local (e.g., backyards), and arguably, impractical across moose habitat. Fernandes et al. (2012) discuss concerns and issues concerning application of entomopathogenic fungi in natural settings for tick control.

ACKNOWLEDGEMENTS

This project was made possible by grants from M. A. Senich (Midland, Texas) to BWN and S. McWilliam to BPS, a gift from E. E. Powelson (Springfield, Ohio) to the Department of Biology at Wittenberg, and the New Hampshire Fish and Game Department (Concord, New Hampshire) to PJP. We thank Y. Guardiola and J. Scott (UAMH, Toronto, Ontario) for fungus identification, J. Benoit (University of Cincinnati, Cincinnati, Ohio) for sequence analysis, and H. Jones and D. Ellingwood of the University of New Hampshire (Durham, New Hampshire) for collecting specimen ticks.

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