Sublethal Effects of *Metarhizium anisopliae* (Deuteromycetes) on Engorged Larval, Nymphal, and Adult *Ixodes scapularis* (Acari: Ixodidae)

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ABSTRACT *Ixodes scapularis* Say adults, nymphs, and larvae were treated with the entomopathogenic fungus *Metarhizium anisopliae* in a combination of field and laboratory experiments to assess sublethal effects of the fungus on *I. scapularis* fecundity and body mass. Postengorgement and egg mass weights were 33 and 50% lower, respectively, in adult females treated with *M. ansiopliae* in the field before engorging on laboratory rabbits. *M. anisopliae* did not significantly reduce egg mass weight, conversion efficiency, or oviposition period in *I. scapularis* females treated with the fungus after engorging on white-tailed deer, although only 33% of treated females oviposited. Engorged nymphs and larvae treated with *M. anisopliae* converted significantly lower percentages of their engorged weight to their molted adult and nymphal weights. This study indicates that *M. anisopliae* reduces fitness (fecundity and body mass) in all active *I. scapularis* stages and indicates that its impact as a biocontrol agent might be higher than that suggested by direct mortality alone.

KEY WORDS biological control, blacklegged tick, entomopathogenic fungus, Lyme disease, tick control

IN NORTH AMERICA, *Ixodes scapularis* Say is the principal vector for *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwald & Brenner (Burgdorfer et al. 1982, Johnson et al. 1984), the bacterium causing Lyme disease, and for the protozoan *Babesia microti* (Spielman et al. 1985) and bacterium *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophila*) (Pancholi et al. 1995, Walker and Dumler 1996, Dumler et al. 2001), which cause babesiosis and human granulocytic ehrlichiosis, respectively. The Centers for Disease Control and Prevention (CDC) reported 23,763 Lyme disease cases nationwide in 2002, continuing a recent trend for growth in incidence (CDC 2003). Because risk of human exposure to these diseases is related to the local abundance of ticks, reducing *I. scapularis* density and population growth is critical to integrated Lyme disease management (White et al. 1991, Daniels and Fish 1995, Ostfeld 1997).

The most effective *I. scapularis* controls include broadcast and barrier applications of chemical pesticides (Schulze et al. 1987, 1991, 1992, 1994, 2000, 2001a; Stafford 1991; Stafford and Kitron 2002, Curran et al. 1993), but these products can have nontarget (Shires 1985, Smith and Stratton 1986, Schulze et al. 2001b) and other negative environmental impacts (Bradbury and Coats 1989, Gassner et al. 1997, Soderlund and Bloomquist 1989, Stark and Banks 2003). As an alternative, biological control agents, such as entomopathogenic fungi (Zhioua et al. 1997, 1999a; Benjamin et al. 2002), nematodes (Zhioua et al. 1995, Hill 1998), and bacteria (Zhioua et al. 1999b) as well as wasp parasitoids (Hu et al. 1993; Stafford et al. 1996, 2003; Knipling and Steelman 2000) have been explored as a method of controlling ticks. The entomopathogenic fungus *Metarhizium anisopliae*, in particular, is lethal to engorged *I. scapularis* larvae and adult females (Zhioua et al. 1997) and controls questing adults (Benjamin et al. 2002). For *I. scapularis*, effectiveness of control agents typically has been measured by percentage mortality of the treated population. However, control agents might reduce fecundity or individual performance (hereafter, fitness) of ticks, with potentially strong impacts on population growth and future abundance (Stark and Banks 2003). Such sublethal effects of either chemical/physical or biological agents on blacklegged ticks have rarely been assessed. Allan and Patrican (1994) reported sublethal effects of desiccants and insecticidal soaps on immature stages of *I. scapularis*, whereas Zhioua et al. (1997) reported no effect of *M. anisopliae* on oviposition of engorged females that survived treatment.

For other species of ixodid ticks, sublethal effects of chemicals (Drummond 1981, 1988; Giles and Rothwell 1983; Kaufman et al. 1986; Wilson et al. 1991;

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Wilson 1993; Bates et al. 1995; El-Azazy and Lucas 1996; Bicalho et al. 2001) and of the entomopathogenic fungi *Aspergillus ochraceus* (Estrada-Pena et al. 1990), *Beauveria bassiana* (Mwangi et al. 1995, Kaaya et al. 1996, Kaaya and Hassan 2000, Gindin et al. 2001, Samish et al. 2001), and *M. anisopliae* have been reported. *M. anisopliae* applied to engorged or engorging adult female ticks reduced fecundity, occlusion rate, preoviposition and oviposition periods, egg incubation period, percentage of females ovipositing, feeding period, or engorged female weight in *Boophilus* (Correia et al. 1998, Bittencourt 2000, Frazzon et al. 2000, Kaaya and Hassan 2000, Gindin et al. 2001, Onofre et al. 2001), *Rhipicephalus* (Mwangi et al. 1995, Kaaya et al. 1996, Samish et al. 2001), and *Amblyomma* (Kaaya et al. 1996). Samish et al. (2001) also reported that *M. anisopliae* reduced molting success of engorged *Rhipicephalus* larvae and nymphs.

In combined field and laboratory experiments, we examined whether *M. anisopliae* at sublethal concentrations reduces fecundity of *I. scapularis* adult females treated pre- and postengorging, reduces engorged nymphal and larval fitness postecdysis, and ultimately reduces *I. scapularis* population growth. Combined with knowledge of lethal effects of *M. anisopliae* on *I. scapularis*, understanding of sublethal effects could lend stronger support to *M. anisopliae* use for *I. scapularis* control and could further elucidate the best application method to use (e.g., broadcast versus host-targeted application), which tick stage to target, and on which tick host to focus.

Materials and Methods

Treatment of Unfed Female *I. scapularis* with *M. anisopliae*. In March 2002, we established six 30 by 30-m plots (20 by 20 m with 10-m buffer), separated by at least 10 m, on forested property at the Institute of Ecosystem Studies (IES; Millbrook, Dutchess County, New York). The plots were located in a mixed hardwood forest, primarily oak, *Quercus* spp., and white pine, *Pinus strobus* L., canopy, with maple-leaved viburnum, *Viburnum acerifolium* L.; barberry, *Berberis thunburgii* D.C.; *Quercus* spp.; and *P. strobus* in the understory. Researchers marked a 1 by 1-m grid through all plots for tick sampling. From 16 to 19 April 2002 (typically within the spring peak in activity of adult *I. scapularis* at IES; Ostfeld et al. 1996), between 9 a.m. and 5 p.m. each day, researchers sampled for pretreatment tick densities. Sampling was performed by dragging a 1 by 1-m white corduroy drag cloth over 15 1-m rows per plot, randomly sampling five rows per plot at a time and randomly rotating among plots to control for weather and time biases. The corduroy cloth and researchers' clothing were checked for ticks after every 5 m of drag sampling, and before leaving or entering a plot to prevent transportation of ticks between plots. Ticks were counted and released. Plots were randomly designated treatment or control by coin toss. During baseline sampling, we found on average three adult ticks per 100 m^2, whereas 10 adults per 100 m^2 is the average density since at least 1995 for adjacent areas (Goodwin et al. 2001, Ostfeld et al. 2001). To more closely approximate the long-term data and standardize abundance across plots, we increased the estimated adult tick density for this study from three to approximately seven adults per 100 m^2 by collecting adult ticks from a nearby area and adding them to each plot.

On 20 April 2002, we sprayed treatment plots with an aqueous solution of *M. anisopliae* spores (10^8 *M. anisopliae* strain ESC1 spores per milliliter, Bio-Blast Termiticide, Village Farms, Eatontown NJ), formulated by mixing two packets of Bio-Blast dry spores with 1.89 liters of tap water. Spore viability was not tested specifically for these experiments and was assumed to approach 100%. Treatment plots were sprayed using a backpack sprayer at a rate of ≈1–1.5 liters of solution per 100 m^2, covering the entire 30 by 30-m area of each plot from the forest floor up to 1.5 m on vegetation. We walked up and back through each plot ≈10–15 times, spraying from side to side in an ≈3-m swath. Control plots were treated in the same manner with water only. The treatment order of plots was randomly determined. On 23 April 2002, the plots were treated again, exactly as described above.

On 24, 25, and 26 April 2002, we collected adults posttreatment following the same protocol as for baseline sampling, described above, except that sampling occurred over the interior 20 by 20-m portion (leaving a 10-m buffer), and ticks were collected; each tick was placed in a separate 16-ml plastic vial covered with mesh fabric secured by a snap-cap with a hole. Vials were placed in multiple humid chambers (≈90% RH), categorized by treatment or control in an incubator at 22°C (photoperiod of 14:10 [L:D]h). On 7 May 2002, female *I. scapularis* were fed on the ears of a New Zealand White rabbit (control and treatment on separate ears), which were then covered with sacs to prevent tick movement. By 15 May 2002, female *I. scapularis* ticks had either engorged or died. Engorged females were weighed, placed in separate vials (with mesh fabric, and cap with hole), incubated in humid chambers as described above, and examined for fungal infection until 11 June 2002, the end of their oviposition period. Each egg mass was weighed on an analytical balance.

Treatment of Engorged *I. scapularis* Females, Nymphs, and Larvae. Between 11 and 20 November 2002, 54 engorged *I. scapularis* adult females were collected from hunter-killed white-tailed deer, *Odocoileus virginianus*, on IES property. Collected females had either naturally just dropped off the deer or were near repletion and easily plucked from the deer hide. On 21 November 2002 each engorged female was randomly designated treatment or control, rinsed with deionized water, dried on clean filter paper, and weighed. Each tick was placed in a petri dish containing filter paper, and 10 μl of 10^6 spores per milliliter of *M. anisopliae* solution (Bio-Blast Termiticide) or deionized (DI) water for control was pipetted onto the dorsal side and 10 μl onto the ventral side of each female. We placed each tick into a separate snap-cap vial, as described above, which was placed in

a humid chamber and incubated at 26°C (photoperiod of 12:12 [L:D] h). Ticks were examined two to three times a week for signs of fungal infection, mortality, and oviposition. When oviposition was complete and the female dead, each egg mass was weighed, and the dead female was removed. Egg masses were checked two to three times a week for eclosion. Once all noneclosed egg masses were either infected with fungus, dried, or otherwise deteriorated, the experiment was ended on 25 March 2003.

In August 2002, 30 and 60 engorged nymphs and larvae, respectively, were collected from white-footed mice, *Peromyscus leucopus;* eastern chipmunks, *Tamias striatus;* short-tailed shrews, *Blarina brevicauda;* and red, *Tamiasciurus hudsonicus*, and gray, *Sciurus carolinensis*, squirrels at IES. Mammalian hosts were live-trapped and housed in the laboratory temporarily in wire mesh cages; replete ticks were collected after they dropped into pans of water placed beneath cages. After being randomly assigned to control and treatment groups, nymphs and larvae, on 14 and 27 August 2002, respectively, were washed with deionized water, dried on filter paper, and weighed. Engorged immature ticks were placed on filter paper in petri dishes and treated with 2–3 μl of a 10^7 spores per milliliter of solution (LC_{50} for engorged larvae; Zhioua et al. 1997) of *M. anisopliae* (Bio-Blast) and DI water (DI water only for control group). Each engorged tick was then placed in a separate snap-cap vial covered with mesh fabric and a cap with hole. Vials were placed in humid chambers, as described above, and incubated at 25°C (photoperiod of 9:15 [L:D] h).

Both larvae and nymphs were examined twice a week for fungal infection, mortality, or molting until they either molted or died (termination on 4 October and 30 September 2002 for engorged nymphs and larvae, respectively). After the newly molted exoskeleton hardened, each tick was weighed. Newly molted adults and nymphs were then processed and examined for *B. burgdorferi* infection by using the direct immunofluorescence assay technique (Ostfeld et al. 2001).

Statistical Methods. Tick stadia and different response variables (e.g., egg mass weight, body weight of newly molted ticks) were analyzed separately. The general hypothesis that *M. anisopliae* affected fitness measures was tested using the Student's *t*-test, for which proportional data were transformed by the arc sine square root to obtain a normal distribution. Owing to the a priori expectation of reduced fitness of fungus-treated ticks, we used one-tailed test statistics.

Results

Field Treatment of Questing Female *I. scapularis* with *M. anisopliae*. We collected a total of 36 adult *I. scapularis* (16 female, 20 male) from control plots compared with 23 (11 female, 12 male) from fungus treatment plots. Using Abbott's formula (corrected % = [1 − (*n* in treatment group after treatment/*n* in control group after treatment)] * 100; Abbott 1925), we calculated that the percentage control (based on the adjusted populations for treatment and control plots) was 36%. Of the 36 control adults collected, three died (8%), not from fungal infection, whereas 12 of 23 (52%) treatment ticks died of *M. anisopliae* infection. In the control and treatment groups 69% (11/36) and 64% (7/11), respectively, of female *I. scapularis* successfully engorged on a rabbit and laid eggs. The average engorged female mass was significantly lower for treatment (mean ± SE, 214 ± 18 mg) than control ticks (284 ± 13 mg; $t = 3.23$, df = 13, $P = 0.003$; Fig. 1). In addition, average egg mass weight was significantly lower for treatment (109 ± 15 mg) than control groups (163 ± 9 mg; $t = 3.13$, df = 10, $P = 0.005$; Fig. 2). The average ratios of egg mass to engorged female mass (conversion efficiency) was not significantly different between treatment (0.51 ± 0.03) and control (0.55 ± 0.02; $t = 1.52$, df = 12, $P = 0.076$).

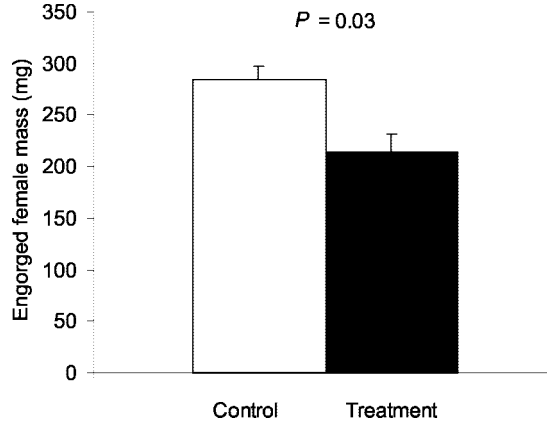

Fig. 1. Engorged mass (mean + 1 SE) of female *I. scapularis* treated with *M. anisopliae* before feeding versus control (untreated) females.

Adult Female *I. scapularis* Treated with *M. anisopliae* Postengorgement. The average mass of engorged females did not differ between control (mean ± SE, 204 ± 11 mg) and treatment (204 ± 11 mg) groups before application of *M. anisopliae* ($t = 0.04$, df = 52, $P = 0.97$). After fungal application, in the control

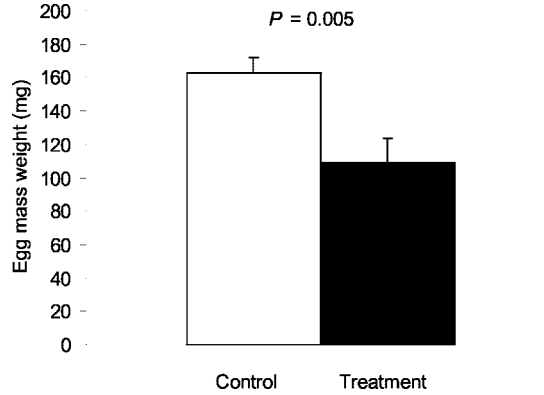

Fig. 2. Egg mass (mean + 1 SE) from engorged female *I. scapularis* treated with *M. anisopliae* before feeding compared with untreated control.

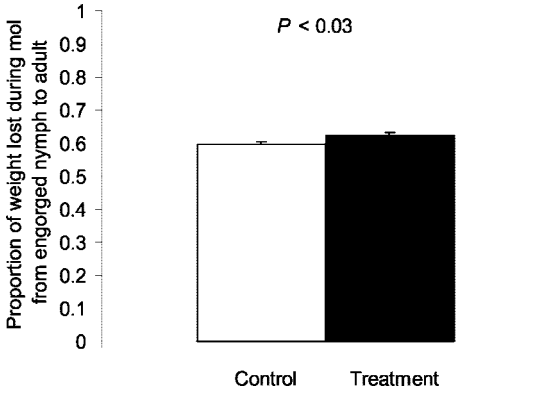

Fig. 3. Proportion of weight lost ([engorged nymphal mass − flat adult mass]/engorged nymphal mass) (mean + 1 SE) by engorged *I. scapularis* nymphs after molting to flat adults, treated with *M. anisopliae* after engorging, versus untreated control.

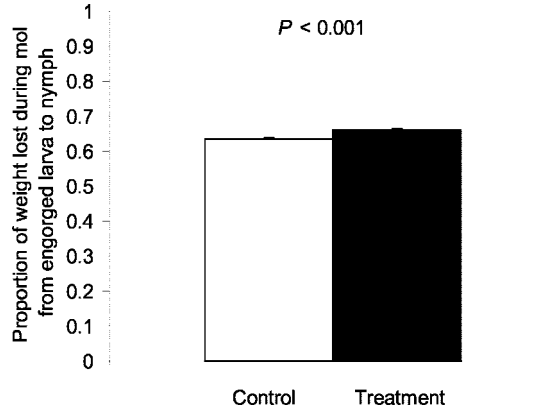

Fig. 4. Proportion of weight lost ([engorged larval mass − flat nymph mass]/engorged larval mass) (mean + 1 SE) by engorged *I. scapularis* larvae after molting to flat nymphs, treated with *M. anisopliae* after engorging versus untreated control.

group, 56% (15/27) of females oviposited, whereas only 33% (9/27) of treatment females oviposited. Length of the preoviposition period did not differ significantly between treatment (32 ± 11 d) and control (41 ± 9 d) groups ($t = 1.94$, df = 14, $P = 0.07$), nor did oviposition period (control mean, 17 ± 2 d; treatment mean, 18 ± 4 d; $t = -0.21$, df = 13, $P = 0.41$). Therefore, total time to postoviposition death was not different between control and treatment groups. Neither egg mass weight (control mean, 28.3 ± 8.0 mg; treatment mean 24.3 ± 11.1 mg; $t = 0.29$, df = 16, $P = 0.38$) nor conversion efficiency (egg mass weight/engorged female weight) differed between control (0.15 ± 0.041) and treatment groups (0.12 ± 0.052; $t = 0.59$, df = 16, $P = 0.28$).

Engorged Nymphal and Larval *I. scapularis* Treated with *M. anisopliae*. Pretreatment weight of engorged *I. scapularis* nymphs did not differ between control (mean ± SE, 2.89 ± 0.28 mg) and treatment (2.93 ± 0.22 mg; $t = -0.11$, df = 24, $P = 0.91$) groups and between *B. burgdorferi*-infected (3.13 ± 0.23 mg) and -uninfected adults (3.18 ± 0.32 mg; $t = 0.13$, df = 17, $P = 0.45$) that molted from these nymphs. In the control group, 87% of engorged nymphs molted, whereas 100% molted in the treatment group. Flat adult *I. scapularis* emerging from treated, engorged nymphs did not differ in mass (treatment mean, 1.10 mg ± 0.09 mg) from those in the control group (mean, 1.18 ± 0.13 mg; $t = 0.47$, df = 23, $P = 0.31$). However, examination of the proportional loss in mass during molt, calculated as (engorged nymphal mass − flat adult mass)/engorged nymphal mass, revealed a significant effect of fungal treatment. Treated *I. scapularis* ticks lost a larger proportion of their engorged nymphal mass after molting to adults (treatment mean, 0.624 ± 0.01; control mean, 0.595 ± 0.01; $t = -2.01$, df = 26, $P = 0.027$; Fig. 3).

Engorged *I. scapularis* larval mass before treatment did not differ between control (mean ± SE, 0.429 ± 0.01 mg) and treatment (0.435 ± 0.01 mg; $t = -0.443$, df = 56, $P = 0.66$) groups and between *B. burgdorferi*-infected (0.438 ± 0.01 mg) and -uninfected groups (0.428 ± 0.01 mg; $t = 0.668$, df = 48, $P = 0.50$). Of the control group, 100% (30/30) larvae survived through molting, whereas 83% (25/30) of treated larvae survived to molt. Flat nymphs that molted from untreated and treated *I. scapularis* larvae did not differ in mass (control mean, 0.157 ± 0.004 mg; treatment mean, 0.148 ± 0.005 mg; $t = 1.52$, df = 47, $P = 0.06$). The treatment group, however, lost a larger proportion of engorged larval mass through molting; that is, less of the engorged larval mass was recovered in the flat nymphal mass, calculated as (engorged larval mass − flat nymphal mass)/engorged larval mass (Fig. 4; treatment mean, 0.660 ± 0.005; control mean, 0.634 ± 0.003; $t = -4.738$, df = 40, $P < 0.001$).

Discussion

Sublethal Effects of *M. anisopliae* on Adult Female *I. scapularis*. After applying aqueous solution of *M. anisopliae* ESC1 (10^8 spores per milliliter) to field sites with known numbers of questing ticks, we retrieved potentially exposed individuals to the laboratory to measure sublethal effects. We found that this entomopathogenic fungus reduced female postengorgement weight and egg mass weight but not the percentage of females ovipositing. Reduction of their conversion efficiency (egg mass weight/engorged female weight) and of the preoviposition period by fungal treatment was not significant ($P = 0.07$ in both cases), but it suggested additional loss of fecundity. Lower postengorgement weight is likely part of a complex host immune response to *M. anisopliae* infection, which can consume host nutrients, suppress host defense, or affect vital host function (such as reproduction; Clarkson and Charnley 1996). Entomopathogenic fungi have reduced feeding in some infected insect hosts as well (Hajek and St. Leger 1994). Given that egg mass weight (or egg number) and engorged female weight correlate positively in ixodid ticks

and that ixodid females rapidly digest and convert almost all of their blood meal into egg production (Oliver 1989, Uspensky and Ioffe-Uspensky 1999), it follows that the treated females (with lower engorged weights) also produced egg masses with lower weights. *M. anisopliae* E9 (10^5–10^8), however, applied to *B. microplus* females engorging on stabled cattle did not decrease engorged female weights (or the percentage of females that oviposited), but in some cases reduced egg mass weight by up to 52% (Correia et al. 1998).

Whereas conversion efficiency (egg mass weight/engorged female weight) was not significantly reduced in our study, a better measure of reproductive efficiency would include percentage of eclosion (e.g., reproductive efficiency calculated as egg mass/engorged female weight * percentage of larvae hatching; Onofre et al. 2001) and hatched larval survival, which we did not examine. *M. anisopliae* reduced engorgement weight, egg mass weight, and egg hatching of *A. variegatum* but not of *R. appendiculatus* adults engorging on rabbits (10^8 spores per milliliter) and of *R. appendiculatus* engorging on cattle (10^9) (Kaaya et al. 1996). In addition, *M. anisopliae* at 10^9 spores per milliliter reduced egg viability by 50% in *B. decoloratus* ticks engorging on Zebu cattle (Kaaya 2000, Kaaya and Hassan 2000). Daniels et al. (1996) found that the single factor distinguishing between successful and unsuccessful reproduction by *I. scapularis* females was engorged female body size. Based on these findings, we would expect *M. anisopliae* to reduce *I. scapularis* egg production, resulting in fewer offspring and a lower population growth rate. Future studies of preengorgement *I. scapularis* females treated with *M. anisopliae* should include examination of *I. scapularis* egg viability as well as of larval survival after eclosion.

We measured additional reproductive parameters after treating engorged female *I. scapularis* females with *M. anisopliae* (10^6 spores per milliliter) and found that *M. anisopliae* did not reduce egg mass weight (in contrast with the preengorgement treatment), conversion efficiency, or oviposition length. The preoviposition period was shorter on average in the treatment group, but this difference was not significant. That only 33% of the treatment group survived to oviposit (compared with 63% in the preengorgement treatment) and that treatment ticks died 55% faster after ovipositing indicate some indirect fitness effects of *M. anisopliae* on engorged *I. scapularis* females. Egg mass weight was not reduced in this experiment probably because of the low *M. anisopliae* concentration used (10^6 compared with 10^8 in the first experiment) or because *I. scapularis* females fed normally, engorging before *M. anisopliae* exposure, thus resulting in egg mass weights positively correlated with engorgement weights, as discussed above. In other studies, higher concentrations of *M. anisopliae* applied to engorged ixodid females reduced egg mass weight. *M. anisopliae* (strain 7 at 10^7 spores per milliliter) applied to engorged *B. annulatus* females significantly reduced fecundity of those surviving fungal infection, reducing egg-laying capacity by >90% and eclosion by 80% (Gindin et al. 2001). *M. anisopliae* strains 7, 43, and 108 (at 10^7 spores per milliliter) applied to engorged *R. sanguineus* females resulted, respectively, in 93, 67, and 100% mortality and 100, 89, and 100% fewer eggs than controls (Samish et al. 2001). When applied to engorged female *B. microplus*, *M. anisopliae* (10^8) increased preoviposition, egg incubation, and eclosion periods, shortened the oviposition period, and decreased eclosion percentage and reproductive efficiency (Bittencourt 2000). For engorged *B. microplus* females treated with *M. anisopliae* strains CG-30 and CG-46 (10^6–10^8 spores per milliliter), Onofre et al. (2001) reported 38–73% and 35–60% eclosion, respectively, and low reproductive efficiencies (egg weight/engorged female weight * percentage of eclosion). Although eclosion was not examined in this study because no eggs hatched in control or treatment groups, based on the literature we might expect lower eclosion (and therefore reproductive efficiency) from eggs of treated, engorged *I. scapularis.* Although our studies indicate that treating *I. scapularis* females after feeding instead of before feeding has weaker sublethal effects, confounding variables preclude certainty.

Sublethal Effects of *M. anisopliae* on Engorged Nymphal and Larval *I. scapularis*. Although we did not find a significant reduction in weights of newly molted adult or newly molted nymphal *I. scapularis* after treating engorged nymphs and larvae, respectively, with 10^7 spores per milliliter of *M. anisopliae*, postmolt both treatment groups on average weighed less than control groups and the difference approached statistical significance ($P = 0.06$) for larvae. Considering the trend for lower postmolt weight in the treatment groups and that treatment groups weighed slightly more when engorged, we found that fungal treatment significantly reduced the proportion of engorged weight lost during ecdysis in the treatment groups. In the control groups, 40% of engorged nymphal and 37% of engorged larval weights were converted to the flat molted stages versus 38 and 34% in treatment groups. These results were significantly different between control and treatment groups in part because of low variance and they suggest that for treated *I. scapularis*, energy not converted to molted weights was otherwise used to fight pathogenic effects of *M. anisopliae.* Molted, treatment ticks, therefore, could be less fit, having less energy to survive without feeding and therefore dying sooner. Samish et al. (2001) found that only 7–11% of engorged *R. sanguineus* larvae and 2.5–10% of engorged nymphs surviving treatment with 10^7 spores per milliliter *M. anisopliae* (strains 7, 43, and 108) emerged as flat nymphs and adults, respectively; 36–75% of emerged nymphs and 62.5–100% of emerged adults died within 5 d, resulting in a nymphal yield of 2–7% from treated engorged larvae and a 0–6% yield for adults from treated engorged nymphs. Given our documentation that *I. scapularis* females treated with *M. anisopliae* before engorging had lower engorgement weights, we might expect similar sublethal effects on flat adults and nymphs surviving *M. anisopliae* treatment as engorged nymphs

and larvae, respectively. Furthermore, we would expect lower postengorgement weights and molting success for larvae and nymphs treated with *M. anisopliae* before engorging.

Our results show that *M. anisopliae* affects fitness of *I. scapularis* adult females, nymphs, and larvae, through sublethal pathways, although studies over longer time periods would be necessary to construct a model predicting population consequences. Strong sublethal effects combined with previously reported lethal effects (Zhioua et al. 1997, Benjamin et al. 2002) strengthen the case for use of *M. anisopliae* in *I. scapularis* control. Further studies should focus on determining which application method or which combination of methods would be most effective and on the net consequences of lethal and sublethal effects for population dynamics of ticks.

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