Effectiveness of *Metarhizium anisopliae* (Deuteromycetes) against *Ixodes scapularis* (Acari: Ixodidae) engorging on *Peromyscus leucopus*

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ABSTRACT: With the incidence of Lyme disease increasing throughout the United States, reducing risk of exposure to the disease is of the utmost concern. In the northeastern U.S., the blacklegged tick, *Ixodes scapularis*, is the primary vector and the white-footed mouse, (*Peromyscus leucopus*), the primary reservoir for *Borrelia burgdorferi*, the bacterium causing Lyme disease. Targeting *I. scapularis* engorging on white-footed mice with an effective biological control agent, such as the fungus *Metarhizium anisopliae*, could be an effective and relatively safe control technique. In 2002-2003, we performed laboratory and field experiments to determine whether *M. anisopliae*-treated nesting material could effectively control larval *I. scapularis* ticks engorging on white-footed mice, and therefore reduce the number of infected nymphal *I. scapularis* questing the following summer. Our laboratory experiment demonstrated a strong negative effect of *M. anisopliae*-treated nesting material on survival *of I. scapularis* larvae feeding on *P. leucopus*, with 75% versus 35% larval mortality in treatment versus control nests. Our field trials caused only modest, localized reductions in nymphal abundance and had no effect on the proportion of nymphal *I. scapularis* infected with *B. burgdorferi*. Field results probably could be improved by increasing the density of nestboxes to allow fungal delivery to a higher proportion of the mouse population and by deploying nestboxes in an area with lower mammalian diversity, such as a suburban landscape. ***Journal of Vector Ecology* 30 (1): 91-101. 2005.**

Keyword Index: Lyme disease, biological control, white-footed mouse, entomopathogenic fungus, tick control.

INTRODUCTION

Throughout the United States, Lyme disease incidence has been steadily increasing, with the Centers for Disease Control and Prevention (CDC) reporting over 23,000 Lyme disease cases nationwide in 2002 (CDC 2003). *Ixodes scapularis* Say is the primary vector in the northeastern United States for the causative agent of Lyme disease, *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwald & Brenner (Burgdorfer et al. 1982, Johnson et al. 1984), as well as for *Babesia microti* Franca (Spielman et al. 1985) and *Anaplasma phagocytophilum* (Dumler et al. 2001, Pancholi et al. 1995, Walker and Dumler 1996), which cause babesiosis and human granulocytic ehrlichiosis, respectively. Through a perpetuating cycle, *I. scapularis* ticks infect and then are reinfected when feeding on *Peromyscus leucopus* (the white-footed mouse), the primary reservoir in the northeast for *B. burgdorferi* (Mather et al. 1989, Schmidt and Ostfeld 2001, Schmidt et al. 1999). Host-seeking, infected *I. scapularis* nymphs pose the greatest human risk of exposure to Lyme disease (Barbour and Fish 1993, Ginsberg 1994). Efforts to reduce exposure risk have been directed at personal protective measures, education, and *I. scapularis* control using broadcast and host-targeted applications of chemical acaricides (Ginsberg 1994). The native soil fungus, *Metarhizium anisopliae,* has also effectively controlled questing *I. scapularis* via broadcast application (Benjamin et al. 2002). Targeting *I. scapularis* engorging on white-footed mice with an effective biological control agent, such as *M. anisopliae*, could be an effective and relatively safe control technique.

Broadcast and barrier synthetic acaricide applications have been the most common *I. scapularis* abatement methods (Stafford 1991, 1997, Schulze et al. 1987, 1991, 1992, 1994, 2000, 2001a, Patrican and Allan 1995, Solberg et al. 1992, Curran et al. 1993, Allan and Patrican 1995), but may be damaging to the environment. Drawbacks to broadcast applications of most chemical pesticides include environmental contamination (Shires 1985, Smith and Stratton 1986, Bradbury and Coats 1989, Gassner et al. 1997, Soderlund and Bloomquist 1989, Stark and Banks 2003), impacts on non-target organisms (e.g., Schulze et al. 2001b, Shires 1985, Smith and Stratton 1986), and, with overuse, the evolution of resistance by target vectors (e.g., Fernandes 2001, Miller et al. 2001, Nolan 1990, Beugnet and Chardonnet 1995, Soderlund and Knipple 2003, Hemingway and Ranson 2000).

As naturally occurring pathogens, biological control agents appear safer but generally less effective than synthetic chemicals (Croft 1990, Johnson et al. 2002, Lacey et al. 2001, Zimmermann 1993, Ginsberg et al. 2002). *M. anisopliae* induced high mortality in engorged *I. scapularis* larvae, engorged females (Zhioua et al. 1997), and unfed adults in the laboratory (Benjamin et al. 2002). High mortality and control of questing adult *I. scapularis* under field conditions were also achieved using a broadcast application of *M. anisopliae* (Benjamin et al. 2002).

More targeted methods of ixodid tick control include chemical applications to hosts. Host-targeted chemical acaricide dipping, pour-on, and systemic treatments have long been practiced to control ixodid pests of livestock (e.g.,

George 2000, Wilson et al. 1991, Ahrens et al. 1988, Drummond 1985). Recently, similar methods have involved self-treatment of white-tailed deer (*Odocoileus virginianus*) with either amitraz or permethrin at self-medicating bait stations (Sonenshine et al. 1996, Pound et al. 2000, Solberg et al. 2003, Carroll et al. 2002). Efforts to control *I. scapularis* on white-footed mice via distribution of permethrin-impregnated cotton, meant for use as mouse nesting material, resulted in little if any effect on questing and infected nymphal *I. scapularis* (Mather et al. 1987, Daniels et al. 1991, Deblinger and Rimmer 1991, Stafford 1991, 1992). Advantages of host-targeted over areal treatment include lower impact on non-target organisms and less residual contamination. A key disadvantage of host-targeted synthetic pesticide use, however, is the evolution of pesticide resistence (e.g., George 2000, Kaaya 2000). In contrast to synthetic pesticides, species used for biocontrol are able to evolve in response to any evolved resistance in ticks. Using biological control agents in a host-targeted approach could prove to be a safe and effective ixodid tick control method, although few have investigated the potential of such techniques for controlling tick vectors of human disease. Significant mortality was achieved by spraying *M. anisopliae* or *Beauveria bassiana* directly onto cattle infested with *Boophilus microplus* (Correia et al. 1998, Kaaya and Hassan 2000), *B. decoloratus* (Kaaya 2000), or *Rhipicephalus appendiculatus* (Kaaya et al. 1996). We are not aware of evidence supporting the evolution of resistance by ticks to biocontrol agents.

In 2002-2003, we conducted separate laboratory and field experiments to determine whether *M. anisopliae*-treated nesting material placed in artificial nests (nestboxes) could effectively control larval *I. scapularis* ticks engorging on white-footed mice, and therefore reduce the number of infected nymphal *I. scapularis* questing the following year. We also examined whether this treatment reduced immature *I. scapularis* burdens on *P. leucopus* during the treatment period and the year following treatment. To determine whether *M. anisopliae* persists in the nestbox environment over the winter following treatment, we sampled and assayed nesting material one year after application for *M. anisopliae* activity.

MATERIALS AND METHODS

Laboratory component: *M. anisopliae*-treated nesting material

In September 2002, 22 white-footed mice (*P. leucopus*) were live-trapped at the Institute of Ecosystem Studies (IES; Millbrook, Dutchess County, New York) and transported to the laboratory. Each mouse was placed in a wire cage (15 x 15 x 50 cm) with 20 g of cotton batting placed in one end, and water and rodent chow in the other end. Eleven cages were randomly designated for treatment and eleven as controls. Prior to placement in the cage, cotton batting was sprayed with 10 ml of either 10^8 spores/ml *M. anisopliae* ESC1 (Bio-Blast Termiticide®, Village Farms, Eatontown NJ) spore solution (for treatment) or cornstarch solution (control). The aqueous *M. anisopliae* spore solution was formulated by mixing one 19 g packet of Bio-Blast dry spores with 250 ml of tap water; the control solution was 19 g of cornstarch with 250 ml tap water. One mouse was placed in each cage, and each cage was secured within a plastic tub lined with double-sided adhesive tape (to prevent ticks from escaping after dropping from mice). Lighting in the room was set to a 14:10 hour light:dark photoperiod. Treatment and control cages were kept on opposite sides of the room, and were shielded with sheets and shower curtains hanging vertically.

I. scapularis ticks dropping from mice were collected every 12 h and placed in 16 ml mesh-ventilated, snap-cap vials specific to each mouse, tick stage, and day collected, with no more than 10 larvae or five nymphs per vial. Vials were kept in multiple humid chambers (at least 90% relative humidity), designated treatment and control, and incubated (25ºC, 14:10 L:D photoperiod). After 72 h, mice were released at their original capture sites. After all ticks were collected, each tick was placed in a separate vial and stored as above. For animals dropping more than 10 ticks each, 10 ticks were randomly selected (with an equal number for each day) for monitoring and each placed in a separate vial. Ticks were monitored every other day until all ticks either died or molted.

For data analysis, proportional *I. scapularis* mortalities were transformed by the arcsine square root to obtain a normal distribution and compared between treatment and control groups using Student's t test. Two animals in the treatment group were removed from analysis because they dropped two or fewer ticks each. Owing to the a priori expectation of higher mortality in ticks from the fungus-treated mice, we used 1-tailed statistics.

Field component: *M. anisopliae* application to mouse nesting material

Field experiments were performed at IES on six long-term study grids, arranged as three pairs, with one member of a pair used as an unmanipulated control and the other as a treatment grid. Each grid was approximately 2.25 hectares; members of a pair were separated by at least 150 m. Five of the six grids were arranged as an 11 by 11 grid and one as a 12 by 10 grid, with stations (also used for trapping) permanently marked every 15 m. Long-term *I. scapularis* densities as well as mammalian host data had been collected on all three treatment grids and one control grid since 1995 and since 1991 on the other two control grids. Habitat consisted of mixed hardwood (primarily oak [*Quercus* spp.], hickory [*Carya* spp.], and white pine [*Pinus strobus*]) canopy, with maple-leaved viburnum (*Viburnum acerifolium*), barberry (*Berberis thunburgii*), *Quercus*, and *P. strobus* understory (see Jones et al. 1998 for details).

Either 20 or 21 nestboxes were installed on each grid in 1995 on trees at least 10 cm diameter at breast height (dbh) at a height of 1.3 m. Nestboxes were placed 15-50 m apart, equaling a nestbox density of about 9 per hectare. Nestboxes were constructed of untreated white pine and painted brown to match tree bark. Each nestbox measured 14 by 15 by 19-23 cm (H) with a sloped roof design; the roof overhung the sides and front and was hinged to allow opening from the top. Lids were tightly closed and securely fastened with steel wire. Each nestbox had two 2.5 cm diameter holes, one on

each side.

In mid-May 2002, just before the first *I. scapularis* larval peak (dates based on long-term data; Ostfeld et al. 1996a), old cotton batting was removed from each nestbox on all 6 grids and replaced with 30 g of fresh cotton batting sprayed on all surfaces with either 10 ml of 10^8 spores/ml *M. anisopliae* ESC1 spore solution (described above) on treatment grids or with 10 ml of cornstarch-water solution (described above) on control grids. At this time, nestboxes were also sampled for mice. During nestbox sampling, new mice were ear-tagged and released, previously tagged mice recorded, and data gathered about life history, reproductive activity, and immature *I. scapularis* loads on head, neck, and ears. Mice were then returned to nestboxes. Nestboxes were retreated every two wk until the end of September 2002 (for a total of 10 treatments), and mice therein were sampled monthly until the end of November 2002. To treat nestboxes when not sampling mice, five ml of spore or control solution was sprayed into each side hole on the nestboxes using a flexible nozzle on a standard garden sprayer.

To calculate the percentage of total mice using nestboxes on each grid and to determine whether fungal treatment deterred mice from using nestboxes, we determined which mice, by tag number, were trapped and found in nestboxes. Removing any duplicate captures and juvenile mice, we divided the number of mice found in nestboxes by the total number of mice trapped to determine the proportion of mice on each grid using nestboxes. We then compared proportions between treatment and control grids using the paired Student's t test and 2-tailed statistics (see Table 1).

Using data collected from live-trapping *P. leucopus* every three wk on all six grids during the treatment period and from nestbox sampling, we compared immature *I. scapularis* burdens on mice from control and treatment grids with the entire grid rather than individual mice as the unit of replication, using the paired Student's t-test. With no expectation that tick burdens would differ between control and treatment grids (see below), we used the 2-tailed statistics (Table 1).

To determine *I. scapularis* questing densities, we performed drag sampling on all six grids. Drag sampling occurred on three rows (450 m total), randomly selected for each collection period, in each of the six grids every three-four wk from May to November to capture peak abundance of every *I. scapularis* active life stage (Ostfeld et al. 1996a). Grid sampling order was randomly determined to prevent weather and time of day biases. Drag sampling was performed between 10 a.m. and 5 p.m. by dragging a 1-m^2 white corduroy cloth slowly along each of the 150 m rows selected. Researchers stopped every 30 m to count and collect ticks from the cloth as well as from researchers' clothing. Ticks were placed in ventilated snap-cap vials.

If summer application of *M. anisopliae* increased mortality of larvae feeding on mice, we expected reduced abundance of questing nymphs the following summer (2003) on treatment grids. We chose two methods for testing impacts of *M. anisopliae* treatment on numbers of questing nymphs. The most straightforward was a simple comparison, using paired t-tests, of 2003 nymphal abundance at their respective seasonal peaks between treatment and control grids, based on drag sampling. However, long-term monitoring of immature tick abundance at IES has revealed strong variation among grids and years, which could reduce our ability to detect an effect of the fungus treatment in any given year. Therefore, we also tested grid-specific abundance of questing nymphs in 2003 as compared to the long-term average for that grid using the Student's t test (and 1-tailed statistics). Our hypothesis was that the ratio of questing nymphs in 2003 to the long-term average would be lower in *M. anisopliae* treatment grids than on control grids (see Table 1).

We calculated long-term average nymphal *I. scapularis* densities during the nymphal peak for each grid over the period 1991-2002 for two of the control grids and 1995-2002 for all the remaining grids. Using a paired Student's t test, we compared the ratios of 2003 peak nymphal densities to long-term peak nymphal densities between treatment and control grids (1-tailed statistics; see Table 1).

To determine nymphal *I. scapularis* densities on a more localized level, during the 2003 nymphal peak we drag sampled a 30 by 30 m^2 area around each of the five nestboxes most frequently visited by mice during the treatment period. Localized nymphal densities were averaged and compared between treatment and control sites using the paired Student's t test (1-tailed statistics; see Table 1). In addition, ratios of localized nymphal densities to long-term nymphal densities for each grid were also compared using the paired Student's t test (1-tailed statistics; see Table 1).

We also determined average nymphal burdens on *P. leucopus* and eastern chipmunks (*Tamias striatus*) separately by grid in 2003, using 2003 live-trapping data as described above for *P. leucopus*. We then calculated the total number of nymphs counted on *P. leucopus* and *T. striatus* for the 2003 live-trapping season and averaged those host totals by grid. We compared these data using the paired Student's t test. With the expectation that nymphal burdens on hosts would be lower in treatment grids compared with control grids, as a reflection of an overall reduction in nymphal *I. scapularis* in treatment grids, we used 1-tailed statistics (see Table 1).

For localized nymphal drag sampling, *I. scapularis* ticks were counted, collected in ventilated vials, and tested for *B. burgdorferi* infection using direct immunofluorescence (DFA) assay (Lane and Burgdorfer 1987, Ostfeld et al. 2001). Because we expected fungal treatment to increase mortality of larvae feeding on *P. leucopus*, we expected the proportion of *B. burgdorferi*-infected nymphs to be lower the year following treatment. The proportions of infected nymphal *I. scapularis* for each grid were compared using the paired Student's t test (1-tailed statistics; see Table 1).

To determine if *M. anisopliae* persisted in nestboxes over the winter following treatment, in 2003 we sampled and cultured cotton nesting material from nestboxes using agar medium and live waxworm larvae. To prepare agar, 30 g of wheat germ, 0.25 g of chloramphenicol, and 1 ml of 1.98% glyphosate (Roundup) were combined and added to 1.0 L of deionized water, autoclaved for 15 min, and filtered through cheesecloth. We then added 15 g of agar and poured the mixture into 60 by 15 mm Petri dishes, which were stored at

Table 1. Measuring the effectiveness of *M. anisopliae* nestbox treatment under field conditions.

Hypotheses: *M. anisopliae* nestbox treatment in 2002 compared with control will:	Response Variable(s)	Statistical Test
Neither attract nor repel mice	Percentage mice using nestboxes (treatment versus control grids)	Paired Student's t test (2-tailed)
Neither increase nor decrease immature tick burdens on mice during the treatment period	Average immature *I. scapularis* burdens on mice (data from live-trapping and nestbox sampling) in treatment versus control grids	Paired Student's t test (2-tailed)
Increase mortality of larvae feeding on mice exposed to treatment, resulting in decreased questing nymphal densities the summer following (2003) on treatment grids	1. Average nymphal densities in 2003 on control versus treatment grids	Paired Student's t-test (1-tailed)
	2. Ratios of average nymphal densities in 2003 to long-term average for each grid, control versus treatment grids	Paired Student's t test (1-tailed)
Increase mortality of larvae feeding on mice exposed to treatment, resulting in decreased questing nymphal densities around most frequently used nestboxes in 2003	1. Average localized nymphal densities in 2003 on control versus treatment grids	Paired Student's t-test (1-tailed)
	2. Ratios of average localized nymphal densities in 2003 to long-term averages for each grid, control versus treatment	Paired Student's t test (1-tailed)
Increase mortality of larvae feeding on mice exposed to treatment, resulting in decreased average nymphal burdens on mouse and chipmunk hosts the summer following treatment	1. Average per host nymphal burdens in 2003 on mice and chipmunks on treatment versus control grids	Paired Student's t test (1-tailed)
	2. Total nymphal burdens on average for mouse and chipmunk hosts in each grid, compared between treatment and control grids	Paired Student's t test (1-tailed)
Increase mortality of larvae feeding on white-footed mice, resulting in a decreased proportion of *B. burgdorferi*-infected nymphs in nestbox-localized areas the summer following treatment (2003)	Ratios of average *B. burgdorferi*-infected nymphs over average nymphal density in nestbox-localized areas for each grid, control versus treatment	Paired Student's t test (1-tailed)

room temperature for 24 h, after which contaminated plates were removed.

Waxworm (*Galleria mellonella*) plates were prepared by rinsing waxworm larvae with 70% ethanol for 15 s followed by rinsing with deionized water for 30 s. Two larvae were placed in each 60 by 15 mm Petri dish with two carrot slices, which had been autoclaved for 15 min.

On 11 June 2003 we gathered (using flame-sterilized forceps) four cotton samples from 10 nestboxes on each of the three treatment and three control grids (60 nestboxes total, 240 samples) and immediately placed two samples from each nestbox on separate agar plates and two on waxworm plates, which were then sealed with tape. Plates were held in sealed plastic containers in the dark at room temperature for approximately four wk and were checked twice a week for fungal growth. Any observed *M. anisopliae* growth was subcultured onto Sabouraud dextrose agar (SDA) plates by using a sterile loop to transfer spores to four SDA plates. Then, each waxworm larva showing *M. anisopliae* growth was sterilized for two min in 70% ethanol and placed on an SDA plate. These were monitored for *M. anisopliae* growth for 14 d.

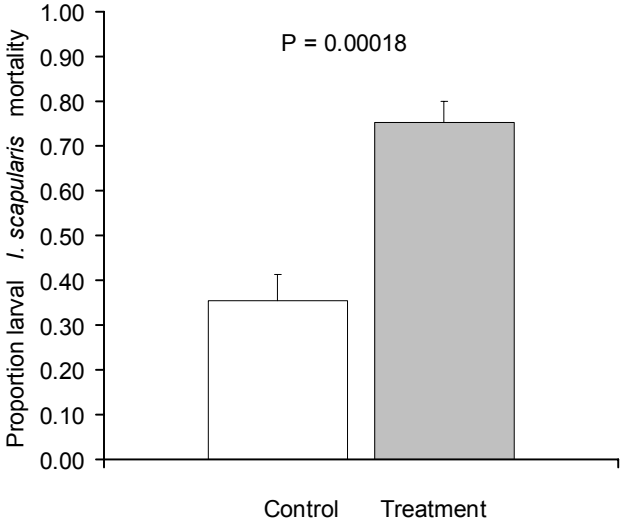

Figure 1. Mortality (±1SE) of engorged *I. scapularis* dropping from mice housed in the laboratory and nesting in *M. anisopliae*-treated (treatment, shaded bar) or in cornstarch-treated (control, open bar) cotton.

RESULTS

Tick larvae that engorged and dropped from mice with *M. anisopliae*-treated nesting material showed 75% mortality. This rate was significantly higher than the 35% mortality observed in engorged larvae dropping from control mice (t = 4.37; df = 18; P = 0.00018; Figure 1).

The presence of *M. anisopliae* solution in nestboxes neither attracted nor repelled mice compared with the same volume of cornstarch solution. The proportions of mice trapped during the treatment period that used nestboxes were similar between treatment (x = 0.345 ± 0.024 SE) and control grids (x = 0.316 ± 0.018 SE; t = -2.835; df = 2; P = 0.105).

During the 2002 treatment period, larval and nymphal *I. scapularis* burdens on live-trapped mice were similar between treatment and control grids (for larval comparison t = -0.725; df = 2; P = 0.544; for nymphal comparison t = -0.856; df = 2; P = 0.482; Figure 2A).

Mice examined from treatment and control nestboxes during the 2002 treatment period were infested with the same average numbers of immature *I. scapularis* ticks (for larval comparison t = 1.115; df = 2; P = 0.381; for nymphal comparison t = 1.498; df = 2; P = 0.273; Figure 2B).

We found that 2003 nymphal densities (nymphs/m^2) on all grids were significantly higher than the long-term average (Figure 3A; t = 4.453; df = 5; P = 0.006). Questing nymphal *I. scapularis* densities in 2003 were not different on average between treatment and control grids (Figure 3B; t = -0.815; df = 2; P = 0.250). Similarly, a comparison of nymphal densities between nestbox-localized areas on treatment and control grids revealed no difference (Figure 3C; t = 1.293; df = 2; P = 0.163). Standardized 2003 nymphal densities (values for 2003 questing nymphal *I. scapularis* for each grid divided by long-term nymphal averages for that grid) for treatment and control grids were not different (Figure 4A; t = 0.391; df = 2; P = 0.367). However, standardized 2003 nymphal densities in the vicinity of nestboxes (nestbox-localized) were significantly lower on *M. anisopliae*-treated grids than on control grids (Figure 4B; t = 3.859; df = 2; P = 0.031).

For the 2003 live-trapping season, the average nymphal *I. scapularis* burdens on mice did not differ between treatment and control grids (for control mice, x = 0.60 ± 0.02 nymphs/mouse; treatment mice x = 0.59 ± 0.12 nymphs/mouse; t = 0.0104; df = 2; P = 0.463). Average nymphal *I. scapularis* burdens on chipmunks were also the same between treatment and control grids (for control chipmunks, x = 3.17 ± 0.72 nymphs/chipmunk; treatment chipmunks x = 3.43 ± 1.24 nymphs/chipmunk; t = -0.483; df = 2; P = 0.338).

The proportion of *B. burgdorferi*-infected *I. scapularis* nymphs in 2003 was not different between nestbox-localized treatment (x = 0.467 ± 0.085) and control grids (x = 0.442 ± 0.033; t = -0.247; df = 2; P = 0.414).

No *M. anisopliae* growth was observed from any treatment or control nestbox cotton samples cultured on agar media, but growth was observed on *G. mellonella* larvae from two different treatment nestboxes. When these samples were subcultured on SDA, *M. anisopliae* grew again in just one instance.

DISCUSSION

I. scapularis host-targeted *M. anisopliae* treatment under laboratory conditions

Laboratory results indicated that *M. anisopliae* application to *P. leucopus* nesting material can effectively control engorging *I. scapularis* larvae. The 75% mortality achieved against engorged larval *I. scapularis* using *M. anisopliae* (10^8 spores/ml) is high, considering the indirect host-targeted methodology, and it is comparable to the 80%

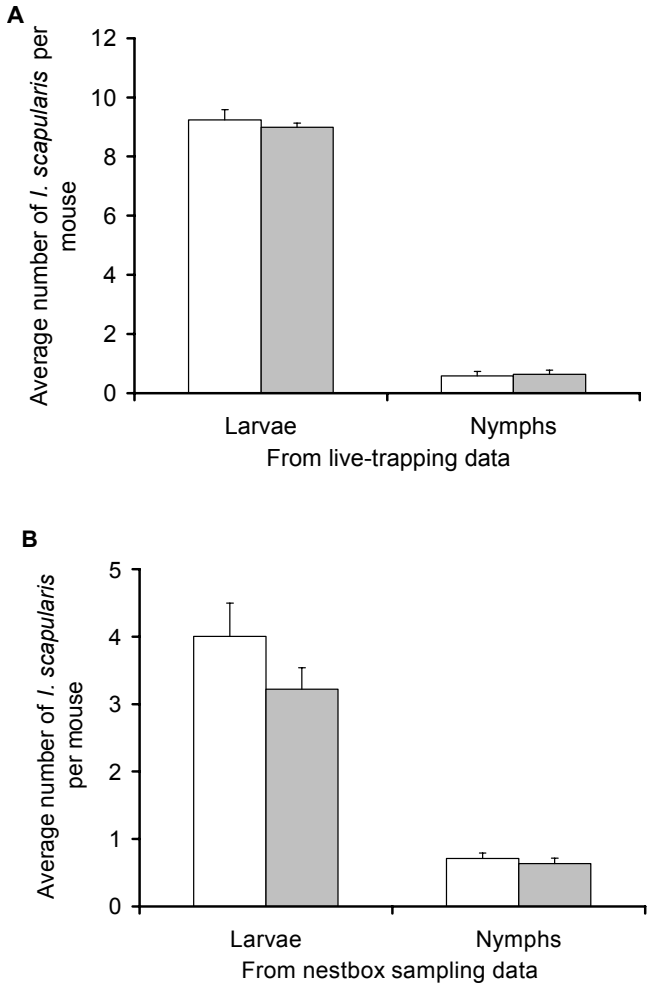

Figure 2. Effects of treating mouse nest boxes with *M. anisopliae* on numbers of immature ticks infesting mice. A. Average immature *I. scapularis* burdens (±1SE) on *P. leucopus* during 2002 fungal treatment period, from live-trapping data. Open, control; shaded, treatment. B. Average immature *I. scapularis* burdens (±1SE) on *P. leucopus* during 2002 fungal treatment period, from nestbox sampling data. Open, control; shaded, treatment.

and 100% mortalities achieved when engorged larval *I. scapularis* were directly submerged in 10^7 and 10^8 spores/ml *M. anisopliae* (strain MABA), respectively, in the laboratory (Zhioua et al. 1997). *M. anisopliae* also proved highly pathogenic to engorged female and unfed adult *I. scapularis* in laboratory experiments (Zhioua et al. 1997, Benjamin et al. 2002) via submersion or spray.

Other ixodid host-targeted treatments with entomopathogenic fungi have focused on livestock tick control by direct fungal application to the host. These experiments have been moderately to highly effective. *M. anisopliae* (isolate E9) at 7.5 x 10^8 spores/ml sprayed onto stabled cattle infested with *B. microplus* larvae induced 40-80% mortalities in female *B. microplus* (Correia et al. 1998) but did not reduce female *B. microplus* density on cattle. *M. anisopliae* (10^9 spores/ml) sprayed onto *R. appendiculatus* naturally infesting Zebu cattle induced 83% mortality (Kaaya et al. 1996). In a

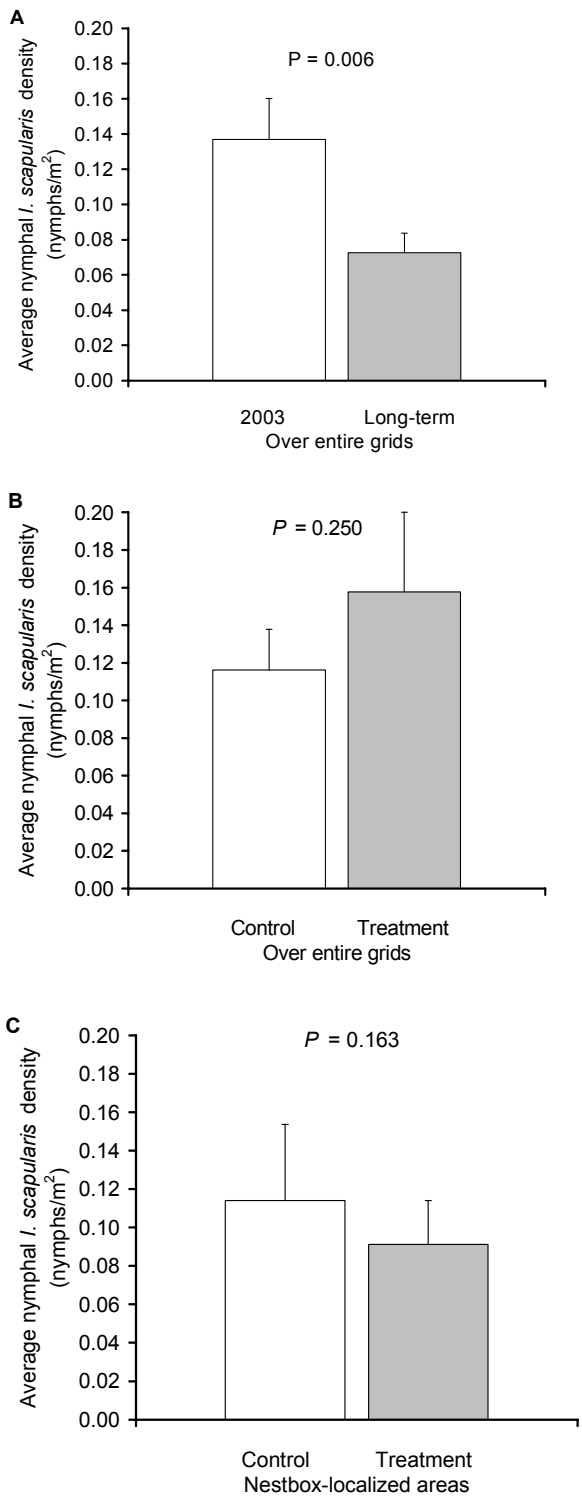

Figure 3. Effects of treating mouse nest boxes with *M. anisopliae* on numbers of host-seeking ticks. A. Average nymphal *I. scapularis* densities (±1SE) (over entire grids) in 2003 compared with the long-term average on all six grids. B. Average nymphal *I. scapularis* density (±1SE) in 2003 (across entire grids) on control versus treatment grids. C. Average nestbox-localized nymphal *I. scapularis* densities (±1SE) in 2003 on control versus treatment grids. Data are from 30 square meter zones surrounding the most frequently visited nestboxes on treatment and control grids.

similar experiment, 10^9 spores/ml *M. anisopliae* solution was sprayed onto *B. decoloratus* on Zebu cattle (Kaaya et al. 1996). After ticks engorged and dropped, they were placed in sealed nylon tetrapacks and placed in grass outside for six wk. Mortality of *B. decoloratus* under these conditions was only 50% (Kaaya 2000). *R. appendiculatus* and *A. variegatum* adults engorging on rabbits under laboratory conditions experienced lower mortalities, however, with 30% and 37% mortality, respectively, when sprayed directly with 5 ml of 10^8 spores/ml *M. anisopliae* (Kaaya et al. 1996).

I. scapularis host-targeted _M. anisopliae_ treatment under field conditions

Under field conditions, host-targeted treatment of *I. scapularis* using *M. anisopliae* did not effectively control *I. scapularis* over a relatively large spatial area but exhibited modest control in smaller, treatment-localized areas. An assumption underlying these studies was that mice would not have an aversion to fungal treatment compared with control; this assumption was supported by data showing that the same percentage (33%) of total mice used nestboxes on treatment and control grids.

We measured immature *I. scapularis* burdens on *P. leucopus* during the treatment period to confirm expectations that *M. anisopliae* does not exhibit repellent effects and that *M. anisopliae* would not impact *P. leucopus* burdens. *M. anisopliae*-induced insect mortality normally requires approximately 5-10 d (St. Leger et al. 1996) and immature *I. scapularis* on *P. leucopus* typically engorge and drop within 72 h (Ostfeld, unpublished data), indicating that any treatment-induced *I. scapularis* mortality would occur several days after the completion of feeding. During the treatment period, larval and nymphal *I. scapularis* burdens on *P. leucopus* were similar between treatment and control grids, as expected (see Figure 2).

By targeting *I. scapularis* larvae engorging on *P. leucopus*, the most competent *B. burgdorferi* reservoir in the northeastern United States (Mather et al. 1989, Schmidt and Ostfeld 2001), we expected to reduce questing nymphal *I. scapularis* density and proportion infected with *B. burgdorferi*, thereby reducing the risk of human exposure to Lyme disease the year following treatment. Treatment did not, however, impact questing nymphal *I. scapularis* densities measured over the entire grid areas in 2003 or the percentage of *B. burgdorferi*-infected nymphs in treatment-localized areas. However, when standardized by the long-term averages on each grid, questing nymphal *I. scapularis* densities were significantly lower in areas around the most frequently used nestboxes in treatment versus control grids.

This modest and localized impact on questing *I. scapularis* nymphs may have resulted from a relatively low percentage of the total population of engorging larvae being exposed to treatment. In the forested landscape at IES, the *I. scapularis* host community is diverse, such that many species feed larval *I. scapularis* and thereby contribute to the next cohort of nymphal *I. scapularis* (LoGiudice et al. 2003). According to the model of Loguidice et al. (2003), at IES *P. leucopus* contributes 14% of questing nymphal *I. scapularis* and 29% of infected nymphal *I. scapularis* per hectare. With just 33% of mice in our grids using nestboxes, the net result is a relatively low number of *M. anisopliae*-treated larvae engorging on *P. leucopus* in our study grids. Stronger results probably could be achieved by deploying nestboxes at a higher density per hectare in an area where host diversity is lower and *P. leucopus* comprises a larger percentage of the host community. Such areas include suburban and urban fragmented landscapes; these areas also tend to have a higher risk of exposure to Lyme disease (Krohne and Hoch 1999, Nupp and Swihart 2000, Falco and Fish 1988, Maupin et al. 1991).

That *M. anisopliae* persisted in one or two treatment nestboxes after the winter season indicates that the nestbox environment is hospitable for *M. anisopliae*, given the protection from ultraviolet light and other elements that have

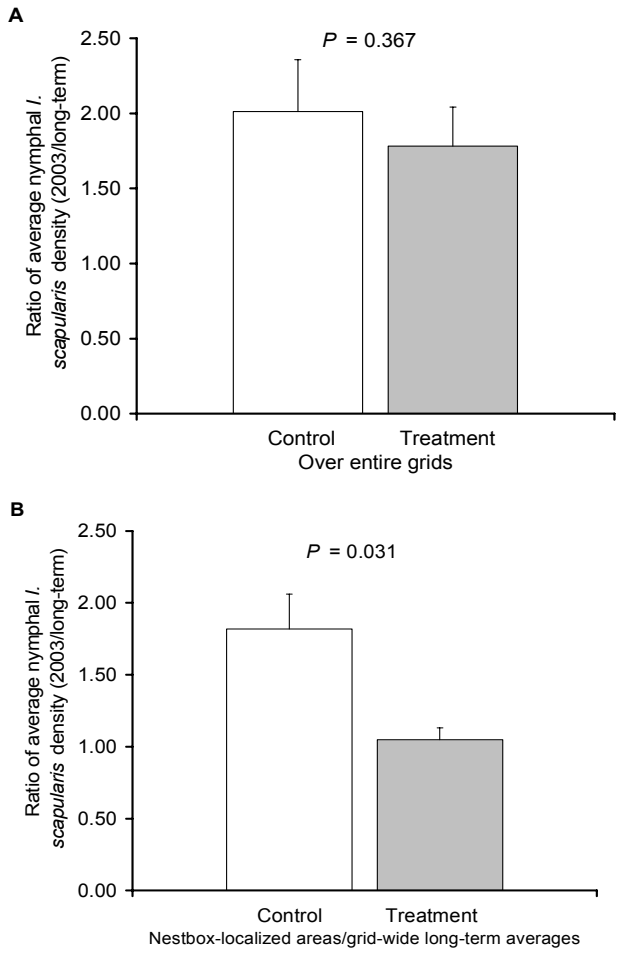

Figure 4. Effects of treating mouse nest boxes with *M. anisopliae* on standardized numbers of host-seeking ticks. A. Ratios (±1SE) of 2003 nymphal *I. scapularis* density (nymphs/m^2) to long-term average nymphal density on control versus treatment grids. Data are from sampling across the entire grids. B. Ratios (±1SE) of 2003 nestbox-localized nymphal *I. scapularis* density (nymphs/m^2) to long-term average nymphal density (across the entire grids) on control versus treatment grids.

reduced the efficacy of entomopathogenic fungi in other applications (Braga et al. 2001).

Other ixodid control efforts targeted at hosts for immature stages have employed synthetic chemical acaricides and have had modest to negative results. Permethrin-impregnated cotton balls deployed in tubes as potential nesting material for ixodid hosts did not reduce the abundance of four species of ixodid ticks of medical importance on dusky-footed woodrats (*Neotoma fuscipes*) (Leprince and Lane 1996). A similar technique reduced *I. scapularis* burdens on *P. leucopus* (Mather et al. 1987, Stafford 1991) but did not impact host-seeking *I. scapularis* densities (Mather et al. 1987, Daniels et al. 1991, Stafford 1991, 1992) or the proportion of ticks infected with *B. burgdorferi* (Daniels et al. 1991, Stafford 1991, 1992); this treatment, therefore, did not reduce the risk of human exposure to infected nymphal *I. scapularis*. In contrast, synthetic chemical acaricides deployed in devices that target white-tailed deer typically have resulted in ≥ 90% control of both *I. scapularis* and *Amblyomma americanum* (Carroll et al. 2002, Pound et al. 2000, Solberg et al. 2003).

Any device designed to kill ticks on free-ranging hosts must attract these hosts to the source of the chemical acaricide or biocontrol agent. Use of a food bait potentially could facilitate population growth in the host, which would undermine the effectiveness of the device if higher host abundance causes higher vector abundance. Impacts of food-based baits on host populations require further study. In contrast, nesting sites for *P. leucopus* do not appear to comprise a limiting resource (Wolff 1994); consequently, we expect no net increase in mouse abundance where nests are provided. Finally, although it is tempting to consider simply eradicating sylvatic mouse populations rather than targeting them with tick control agents to reduce risk of human exposure to infected ticks, we caution against this alternative. Eradication efforts in forests are unlikely to be feasible. Even sustained, aggressive removal-trapping of mice at our sites resulted in only temporary reduction in density (Jones et al. 1998); within weeks of the suspension of this effort, mouse populations returned to levels similar to unmanipulated control areas. Moreover, mice serve crucial ecological functions, such as controlling populations of gypsy moths (*Lymantria dispar*) (Ostfeld et al. 1996b) and supporting populations of hawks and owls (Schmidt and Ostfeld 2003).

In conclusion, our laboratory trial demonstrated a strong negative impact of *M. anisopliae*-treated nesting material on survival of *I. scapularis* larvae feeding on white-footed mice. However, our field trials did not support the hypothesis that summer application of *M. anisopliae* to nestboxes in the field would reduce abundance and infection prevalence of nymphal *I. scapularis* the following year. Only modest, localized reductions in nymphal abundance, compared to long-term averages for the site, were observed. A higher density of nestboxes (>>9 ha^{-1}), which would probably result in delivery of fungus to a higher proportion of the mouse population, would probably increase control. In addition, nestbox treatment of *M. anisopliae* in suburban forests, where vertebrate communities are generally of lower diversity and more dominated by white-footed mice (Allan et al. 2003, LoGiudice et al. 2003), is likely to produce stronger control than we observed in extensive, highly diverse forest habitat.

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