Laboratory and Field Evaluation of the Entomopathogenic Fungus *Metarhizium anisopliae* (Deuteromycetes) for Controlling Questing Adult *Ixodes scapularis* (Acari: Ixodidae)

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ABSTRACT Unfed adult *Ixodes scapularis* Say were treated with spores of the entomopathogenic fungus *Metarhizium anisopliae* Metschnikoff in the laboratory and in the field. An *M. anisopliae* suspension containing 4×10^9 spores per milliliter caused 96% mortality in the laboratory, versus 53% mortality among field-treated ticks. The LC_{50} value for unfed adult *I. scapularis* in the laboratory was 4×10^7 spores per milliliter. Our results indicate that *M. anisopliae* was highly pathogenic to unfed adult ticks and showed potential for controlling questing adult *I. scapularis*.

KEY WORDS *Metarhizium anisopliae*, *Ixodes scapularis*, biological control, blacklegged tick, Lyme disease, entomopathogenic fungi

Ixodes scapularis SAY is the principal vector in North America of *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwald & Brenner (Burgdorfer et al. 1982, Johnson et al. 1984), *Babesia microti* Franca (Spielman et al. 1985), and an as yet unnamed *Ehrlichia* species (Pancholi et al. 1995, Walker and Dumler 1996); the pathogens that cause Lyme disease, babesiosis, and human granulocytic ehrlichiosis, respectively. The prevalence of Lyme disease and other tick-borne diseases can be attributed to a range of factors, with increasing tick population size and the geographic spread of *I. scapularis* being of paramount importance (White et al. 1991, Daniels et al. 1993, Ostfeld 1997). Reducing the abundance of *I. scapularis* is therefore of fundamental importance in managing the incidence and spread of tick-borne illnesses.

Control of *I. scapularis* is based primarily on chemical treatment, which has proven effective against *I. scapularis* adults (Schulze et al. 1987, 1992) and nymphs (Schulze et al. 1991, 1994, 2000, 2001a; Stafford 1991; Curran et al. 1993). However, chemical control has potentially high ecological costs, including damage to nontarget organisms (Schulze et al. 2001b). Ticks are also likely to develop pesticide resistance. Other control alternatives studied previously include habitat modification (Mather et al. 1993, Schulze et al. 1995, Stafford et al. 1998) and management of white-tailed deer populations (*Odocoileus virginianus* Zimmerman) (Wilson et al. 1984, 1988; Daniels and Fish 1993; Deblinger et al. 1993). The effectiveness of controlling *I. scapularis* populations by habitat modification is variable, and habitat modification can have adverse environmental consequences (Ginsberg 1994; Schulze et al. 1995). Reducing deer populations does not appear to be a practical means of reducing Lyme disease risk over large areas (Daniels et al. 1993). Although *I. scapularis* do not remain abundant in the absence of white-tailed deer (Wilson et al. 1988), near eradication of the deer herd is required to significantly reduce tick populations (Wilson et al. 1984, Van Buskirk and Ostfeld 1995).

Given the limitations of pesticide use, deer management, and habitat modification strategies, biological control of *I. scapularis* warrants further attention. Among the biological control candidates studied to date are vertebrate predators (Duffy et al. 1992, Ostfeld and Lewis 1999), entomopathogenic nematodes (Zhioua et al. 1995, Hill 1998), a wasp parasitoid (Hu et al. 1993, Stafford et al. 1996, Knipling and Steelman 2000), entomopathogenic bacteria (Zhioua et al. 1999b), and entomopathogenic fungi (Zhioua et al. 1997, 1999a). Laboratory trials have shown that the entomopathogenic fungus *Metarhizium anisopliae* Metschnikoff is highly pathogenic to engorged larval and engorged adult female *I. scapularis* (Zhioua et al. 1997). In this study, we investigate the potential for *M. anisopliae* to control questing adult *I. scapularis* when the fungus is sprayed on forest understory vegetation.

Materials and Methods

Laboratory Methods. Questing adult *I. scapularis* were collected from vegetation at the Institute of Ecosystem Studies in Millbrook, NY. Ticks were then treated with four *M. anisopliae* spore suspensions at

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concentrations ranging from 4×10^6 to 4×10^9 spores per milliliter. The suspensions were formulated using a commercial preparation of *M. anisopliae* spores (strain ESC 1: Bio-Blast Biological Termiticide). We combined 1.9 liters of tap water with 19 g of dry product to yield a suspension containing 4×10^9 spores per milliliter, and then used serial dilution.

In each trial, ten ticks (five male and five female) were placed on filter paper in a 10 by 1.5-cm petri dish and sprayed with 2.8 ml of spore suspension using a hand sprayer. The same volume of water only was sprayed on control ticks. Trials at each concentration, including the control, were repeated five times (50 ticks at each concentration, 250 ticks total). Immediately after treatment, ticks were placed in clean snap-cap vials, 10 ticks per vial (caps had holes covered with screening). Vials were incubated for 4 wk in a humid chamber at 90% RH and 23°C. The high relative humidity is required for optimal survival of both *I. scapularis* (Daniels et al. 1989) and *M. anisopliae* (Daoust and Roberts 1983). Ticks were examined weekly and mortality was recorded. The LC_{50} (concentration required to kill 50% of ticks) was evaluated after 4 wk using the inhibition concentration approach (Lewis et al. 1994).

Field Methods. We conducted fieldwork at the Institute of Ecosystem Studies between 6 and 19 November 2000, during the peak activity period for adult *I. scapularis* at the study site (Ostfeld et al. 1996). The site features level terrain, a mixed hardwood/white pine (*Pinus strobus*) canopy, and a shrub layer consisting primarily of hardwood saplings interspersed with maple-leaved viburnum (*Viburnum acerifolium*) and patches of barberry (*Berberis thunburgii*). Leaves had fallen from most deciduous species before the beginning of the study period. High *I. scapularis* densities have been recorded at adjacent study sites since at least 1995 (Ostfeld et al. 2001).

We established an experimental grid of 20 plots, each measuring 10 by 10 m, with a minimum of 10 m separating adjacent plots. Moving through the grid in a predetermined order, we used a coin flip to designate each plot as either treatment or control.

On 6 November 2000, drag sampling was used to establish a baseline count of adult ticks occurring in each plot. The entire area of each plot was covered by dragging a 1-m^2 white corduroy drag cloth slowly along 10 directly adjacent parallel transects. At the end of each transect, all adult ticks attached to the drag cloth and to researchers' clothing were counted and immediately replaced on the leaf litter along the same transect. Clothing and drag cloths were carefully examined before leaving and entering plots so as to avoid transporting ticks from plot to plot, or from outside to inside plots.

The number of adult ticks counted during baseline sampling ranged from 1 to 8 per plot, with an average of 4.1 ticks per 100 m^2. To establish treatment and control populations suitable for study, and to minimize variation in starting conditions, we imported ticks from nearby vegetation, increasing tick density to an estimated 10 ticks per 100 m^2 in all plots. This fell within the range of adult tick densities recorded concurrently at nearby sites. Imported ticks were placed individually in the plots on the leaf litter, avoiding edges and areas lacking a shrub layer.

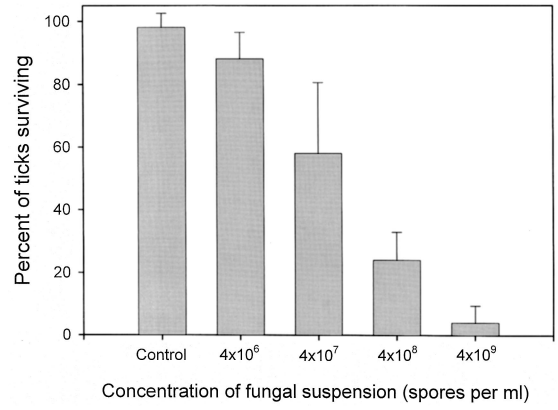

Fig. 1. Percent of adult *I. scapularis* surviving 4 wk after fungal bioassays as a function of spore concentration.

On 13 November 2000, we sprayed the 10 treatment plots with an *M. anisopliae* suspension containing 4×10^9 spores per milliliter. Researchers passed through each plot approximately five times, using an 8-liter capacity sprayer to mist vegetation in 2-m wide swaths. Spray was concentrated on vegetation below the 1.5 m estimated maximum questing height for adult *I. scapularis.* The suspension was applied at a rate of 1.0–1.5 liters/100 m^2, with the variation due primarily to differences in the amount of targeted vegetation present in each plot. Control plots were sprayed in the same manner using water only.

We repeated drag sampling of all plots on 19 November 2000. Collected ticks were counted and placed in 16-ml capped specimen vials. All ticks collected from a given plot were placed in the same vial. Ticks were then incubated in a humidity chamber and monitored weekly for 4 wk. Fungi were subcultured from infected ticks on Sabaroud dextrose agar (Zhioua et al. 1997).

Statistical Analysis. In the field study, the proportion of ticks that died from *M. anisopliae* infection varied from plot to plot. These proportions, or mortality rates, did not follow a normal distribution. The data remained non-normal after log and arc sine square-root transformation. We therefore used a nonparametric test (a one-tailed Mann-Whitney *U* test) to evaluate the differences between mortality rates observed in treatment versus control plots.

Results

Laboratory Results. *M. anisopliae* was highly pathogenic to unfed adult *I. scapularis* in the laboratory. Mortality rates increased with increasing spore concentration (Fig. 1), with 96% (SE ± 0.055) mortality occurring at a concentration of 4×10^9 spores per milliliter. The LC_{50} after 4 wk was 4×10^7 spores per

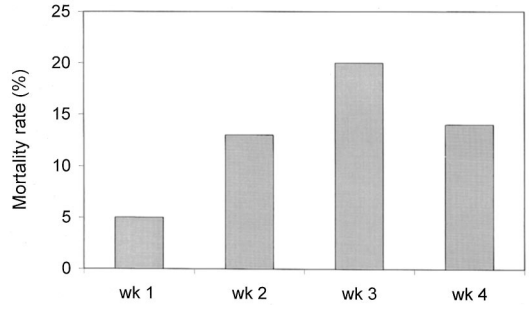

Fig. 2. Weekly mortality rates for adult *I. scapularis* collected from vegetation after fungus treatment.

milliliter. Mortality within the control group was 2% (SE ± 0.045). All dead treated ticks were covered with fungal mycelia, but no fungal growth was observed on the control ticks.

Field Results. Six days after spraying vegetation, we collected 76 adult ticks from treatment plots, versus 92 from control plots. The number of ticks collected from individual plots varied, ranging from 3 to 14 in the treatment group ($\mu = 7.6$, SE ± 4.5), and from 6 to 13 in the control group ($\mu = 9.2$, SE ± 2.0).

After 4 wk in the laboratory, mortality within the treatment group was 53% (40/76), versus 3% in the control group (3/92). Mortality was significantly higher among treated ticks ($U = 90.5$, df = 1, $P = 0.002$). The highest mortality rate occurred between weeks 2 and 3 (Fig. 2). *M. anisopliae* was subcultured from treated ticks, but not from control ticks.

Discussion

Pathogenicity of *M. anisopliae* to Adult *I. scapularis*. Our results demonstrated that *M. anisopliae* (strain ESC1) was highly pathogenic to unfed adult *I. scapularis*. Surface mycosis observed on treated ticks, but not on controls, and the isolation of *M. anisopliae* from field-treated ticks indicated that the fungus was the cause of death in laboratory and field trials.

In the laboratory, tick mortality was positively related to *M. anisopliae* spore concentration, as demonstrated previously with engorged larval and adult *I. scapularis* (Zhioua et al. 1997). A spore concentration of 10^6 spores per milliliter had a low effect, whereas a concentration of 10^7 spores per milliliter induced nearly 50% mortality. Similar results have been reported for *Boophilus microplus* Canestrini (Correia et al. 1998; Bittencourt 2000; Frazzon et al. 2000). Spore density must reach a certain threshold for an effective penetration of the tick's cuticle and subsequent death of the host (Zhioua et al. 1997).

A high spore concentration (4×10^9 spores per milliliter) was required to induce almost 100% mortality among unfed adult *I. scapularis*. In a comparable laboratory study, a concentration of only 10^7 spores per milliliter induced 100% mortality among engorged adult females (Zhioua et al. 1997). Similarly, the mortality rates of unfed and engorged adult *Rhipicephalus appendiculatus* Neumann with the same strain of *M. anisopliae* at 10^6 spores per milliliter were 35 and 81%, respectively (Mwangi et al. 1995). It therefore appears that stretching of the cuticle during engorgement facilitates fungal infection.

In the field, we recorded a mortality rate of 53% among ticks collected from vegetation sprayed with an aqueous formulation of *M. anisopliae*. Other field studies have yielded similar results. Unfed adult *R. appendiculatus* treated with an aqueous formulation of *M. anisopliae* at a concentration of 10^9 spores per milliliter, then placed in potted grass in tetrapacks in the field for 5 wk, had 64% mortality (Kaaya et al. 1996). The same experiment using an oil formulation of *M. anisopliae* showed a mortality rate of 80% (Kaaya 2000). Aqueous formulations of *M. anisopliae* thus seem capable of inducing >50% mortality among questing adult ticks when sprayed on vegetation, and using this fungus in oil formulation could improve the level of tick control.

Multiple applications of *M. anisopliae* during the tick season could also improve the level of control. In one field study, 5-acre paddocks containing Zebu cattle were seeded with larvae of *R. appendiculatus*, then sprayed once a month for 5 mo with an aqueous suspension of *M. anisopliae* at a concentration of 10^8 spores per milliliter. Control paddocks were sprayed with water (Kaaya 2000). After 6 mo, the average numbers of adult *R. appendiculatus* per animal in the treated and control paddocks were 5 and 25, respectively (Kaaya 2000).

The 53% mortality rate among field-treated ticks in this study should be interpreted with caution. Some of the collected ticks dying in vials under optimal conditions for fungal growth might not have died under field conditions. Placing all ticks collected from a given plot in the same vial could have caused horizontal infection from infected to noninfected ticks (Kaaya and Okech 1990), causing an upward bias in the mortality rate. We treated a total area of 1,000 m^2 in this study, worked with a moderate pretreatment tick density of 10 ticks per 100 m^2, and only observed ticks collected 6 d after treatment. Further study will be required to measure the degree of tick control exerted by *M. anisopliae* over time, at varying initial densities, and at larger spatial scales.

Prospects for Biological Control of Questing Adult *I. scapularis*. The acaricidal activity of *M. anisopliae* under field conditions suggests that this fungus could be used in integrated pest management programs to control *I. scapularis*. Particularly promising is the ability of *M. anisopliae* to penetrate the cuticle of questing adult ticks when sprayed on vegetation. Unlike entomopathogenic nematodes, which enter through the everted genital pore of engorged female *I. scapularis* (Zhioua et al. 1995), fungi penetrate directly through the cuticle using enzymatic and/or physical mechanisms (St. Leger 1993).

Our weekly mortality data indicated that *M. anisopliae* typically requires at least 2 wk to invade and kill questing adult *I. scapularis*. This lengthy infection process could allow infected ticks to inoculate uninfected

ticks while co-infesting mammalian hosts. During peak activity periods, adult ticks cluster on the head, ears, neck, and chest of white-tailed deer (Schmidtmann et al. 1998), where the warm, humid, and crowded conditions are favorable for the growth and transmission of *M. anisopliae.* To further assess the biocontrol potential of *M. anisopliae,* it will therefore be important to determine whether mycotic infection is transmitted horizontally on tick hosts, and whether engorgement of *I. scapularis* subsequent to fungus exposure enhances the pathogenicity of the fungus.

It will also be important to assess the pathogenicity of different species and different strains of entomopathogenic fungi against *I. scapularis.* The pathogenicity of five species of entomopathogenic fungi including *Beauvaria bassiana* Balsamo, *M. anisopliae, Metarhizium flavoviridae* Gams & Rozsypal, *Paecilomyces fumosoroseus* Wize, and *Verticillium lecanii* Zimmermann have been tested against engorged female *Boophilus annulatus* Say using a concentration of 10^7 spores per milliliter (Gindin et al. 2001). These authors showed that *M. anisopliae* is the most virulent species, with mortality rates for *M. anisopliae* strain 7 and strain 43 recorded as 90 and 60%, respectively (Gindin et al. 2001). Frazzon et al. (2000) tested the pathogenicity of 12 isolates of *M. anisopliae,* and showed that the strain E6S1 is the most pathogenic against engorged female *B. microplus,* with an LC_{50} of 10^5 spores per milliliter. The mortality rates for *Amblyomma americanum* F. and *R. appendiculatus* with the same strain of *M. anisopliae* at the same concentration were 35 and 83%, respectively (Kaaya et al. 1996). Clearly, susceptibility of ticks to entomopathogenic fungi differs for different species of ticks and fungi.

Curbing *I. scapularis* populations is a cornerstone for controlling *I. scapularis*-transmitted diseases. Targeting adult *I. scapularis* can result in a dramatic reduction in tick fecundity and subsequent tick density. Some recent *I. scapularis* control efforts have been directed at the parasitic adult stage when feeding on deer, using self-treatment devices (Sonenshine et al. 1996, Pound et al. 2000). Because *I. scapularis* is a three-host tick that spends most of its life in soil and on vegetation, the development of safe and effective methods for controlling free-living ticks is also warranted.

The use of natural enemies, such as entomopathogenic fungi, is generally perceived to be ecologically preferable to chemical treatment for controlling pests. However, biological control carries its own risks (Simberloff and Stiling 1996), including the potential for damage to nontarget organisms. Nontarget effects may be less of a concern when native organisms are used for biological control (Goettel and Hajek 2001). *M. anisopliae* occurs naturally in soils in the northeastern United States (Humber 1992), but it has a broad host range, with insect species from seven orders known to be affected (Zimmerman 1993). Further study of the possible nontarget effects of *M. anisopliae,* and means of reducing these effects, is therefore needed if the ecological risks are to be weighed against the expected benefits of *I. scapularis* population control.

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References Cited

Bittencourt, V.R.E.P. 2000. Trials to control South American ticks with entomopathogenic fungi. Ann. N.Y. Acad. Sci. 916: 555–558.

Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis. 1982. Lyme disease—a tick-borne spirochetosis? Science 216: 1317–1319.

Correia, A.C.B., A. C. Fiorin, A. C. Monteiro, and C. J. Verissimo. 1998. Effect of *Metarhizium anisopliae* on the tick *Boophilus microplus* (Acari: Ixodidae) in stable cattle. J. Invertebr. Pathol. 71: 189–191.

Curran, K. L., D. Fish, and J. Piesman. 1993. Reduction of nymphal *Ixodes dammini* (Acari: Ixodidae) in a residential suburban landscape by area application of insecticides. J. Med. Entomol. 30: 107–113.

Daniels, T. J., and D. Fish. 1993. Effect of deer exclusion on the abundance of immature *Ixodes scapularis* (Acari: Ixodidae) parasitizing small and medium-sized mammals. J. Med. Entomol. 32: 5–11.

Daniels, T. J., D. Fish, and R. C. Falco. 1989. Seasonal activity and survival of adult *Ixodes dammini* (Acari: Ixodidae) in southern New York State. J. Med. Entomol. 26: 610–614.

Daniels, T. J., D. Fish, and I. Schwartz. 1993. Reduced abundance of *Ixodes scapularis* (Acari: Ixodidae) and Lyme disease risk by deer exclusion. J. Med. Entomol. 30: 1043–1049.

Daoust, R. A., and D. Roberts. 1983. Virulence of natural and insect-passaged strains of *Metarhizium anisopliae* to mosquito larvae. J. Invertebr. Pathol. 39: 107–117.

Deblinger, R. D., M. L. Wilson, D. W. Rimmer, and A. Spielman. 1993. Reduced abundance of immature *Ixodes dammini* (Acari: Ixodidae) following incremental removal of deer. J. Med. Entomol. 30: 144–150.

Duffy, D. C., R. Downer, and C. Brinkley. 1992. The effectiveness of helmeted guineafowl in the control of the deer tick, the vector of Lyme disease. Wilson Bull. 104: 342–345.

Frazzon, A.P.G., I.S.V. Junior, A. Masuda, A. Schrank, and M. H. Vainstein. 2000. In vitro assessment of *Metarhizium anisopliae* isolates to control the cattle tick *Boophilus microplus.* Vet. Parasitol. 94: 117–125.

Gindin, G., M. Samish, E. Alekseev, and I. Glazer. 2001. The susceptibility of *Boophilus annulatus* (Ixodidae) ticks to entomopathogenic fungi. Biocontrol Sci. Technol. 11: 111–118.

Ginsberg, H. S. 1994. Lyme disease and conservation. Conserv. Biol. 8: 343–353.

Goettel, M. S., and A. E. Hajek. 2001. Evaluation of nontarget effects of pathogens used for management of arthropods, pp. 81–94. *In* E. Wajnberg, J. K. Scott, and P. C.

Quimby (eds.), Evaluating indirect ecological effects of biological control. CAB, New York

Hill, D. E. 1998. Entomopathogenic nematodes as control agents of developmental stages of the black-legged tick, *Ixodes scapularis.* J. Parasitol. 84: 1124–1127.

Hu, R., K. E. Hyland, and T. N. Mather. 1993. Occurrence and distribution in Rhode Island of *Hunterellus hookeri* (Hymenoptera: Encyrtidae), a wasp parasitoid of *Ixodes dammini.* J. Med. Entomol. 30: 277–280.

Humber, R. A. 1992. Collection of entomopathogenic fungal cultures: catalog of strains. Publ. No. ARS-110. U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD.

Johnson, R. C., F. W. Hyde, G. P. Schmid, A. G. Steigerwalt, and D. J. Brenner. 1984. *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. Int. J. Syst. Bacteriol. 34: 496–497.

Kaaya, G. P. 2000. Laboratory and filed evaluation of entomogenous fungi for tick control. Ann. N.Y. Acad. Sci. 916: 559–564.

Kaaya, G. P., and M. A. Okech. 1990. Horizontal transmission of mycotic infection in adult tsetse, *Glossina morsitans morsitans.* Entomophaga 35: 589–600.

Kaaya, G. P., E. N. Mwangi, and E. A. Ouna. 1996. Prospects for biological control of livestock ticks, *Rhipicephalus appendiculatus* and *Amblyomma variegatum,* using the entomogenous fungi *Beauvaria bassiana* and *Metarhizium anisopliae.* J. Invertebr. Pathol. 67: 15–20.

Knipling, E. F., and C. D. Steelman. 2000. Feasibility of controlling *Ixodes scapularis* ticks (Acari: Ixodidae), the vector of Lyme disease, by parasitoid augmentation. J. Med. Entomol. 37: 645–652.

Lewis, P., D. Klemm, J. Lazorchak, T. Norbert-King, M. Heber, and W. Peltier. 1994. Short-term methods for testing the chronic toxicity of effluents and surface waters to freshwater organisms, 2nd ed. EPA-600/4–91–003. Environmental Monitoring Systems Laboratory, Cincinnati, OH.

Mather, T. N., D. C. Duffy, and S. R. Campbell. 1993. An unexpected result from burning vegetation to reduce Lyme disease transmission in ticks. J. Med. Entomol. 30: 642–645.

Mwangi, E. N., G. P. Kaaya, and S. Essuman. 1995. Experimental infections of the tick *Rhipicephalus appendiculatus* with entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae,* and natural infections of some ticks with bacteria and fungi. J. Afr. Zool. 109: 151–160.

Ostfeld, R. S. 1997. The ecology of Lyme disease risk. Am. Sci. 85: 338–346.

Ostfeld, R. S., and D. N. Lewis. 1999. Experimental studies of interactions between wild turkeys and black-legged ticks. J. Vector Ecol. 24: 182–186.

Ostfeld, R. S., K. R. Hazler, and O. M. Cepeda. 1996. Temporal and spatial dynamics of *Ixodes scapularis* (Acari: Ixodidae) in a rural landscape. J. Med. Entomol. 33: 90–95.

Ostfeld, R. S., E. M. Schauber, C. D. Canham, F. Keesing, and J. O. Wolff. 2001. Effects of acorn production and mouse abundance on abundance and *Borrelia burgdorferi* infection prevalence of nymphal *Ixodes scapularis* ticks. Vector Borne Zoonotic Dis. 1: 55–63.

Pancholi, P., C. P. Kolbert, P. D. Mitchell, K. D. Reed, J. S. Dumler, J. S. Bakken, S. R. Telford III, and D. H. Persing. 1995. *Ixodes dammini* as a potential vector of human granulocytic ehrlichiosis. J. Infect. Dis. 172: 1007–1012.

Pound, J. M., J. A. Miller, and J. E. George. 2000. Efficacy of amitraz applied to white-tailed deer by the '4-Poster' topical treatment device in controlling free-living lone star ticks (Acari: Ixodidae). J. Med. Entomol. 37: 878–884.

Schmidtmann, E. T., J. F. Carroll, and D. W. Watson. 1998. Attachment-site patterns of adult blacklegged ticks (Acari: Ixodidae) on white-tailed deer and horses. J. Med. Entomol. 35: 59–63.

Schulze T. L., W. M. McDevitt, W. E. Parkin, and J. K. Shisler. 1987. Effectiveness of two insecticides in controlling *Ixodes dammini* (Acari: Ixodidae) following an outbreak of Lyme disease in New Jersey. J. Med. Entomol. 24: 420–424.

Schulze, T. L., G. C. Taylor, R. A. Jordan, E. M. Bosler, and J. K. Shisler. 1991. Effectiveness of selected granular acaricide formulations in suppressing populations of *Ixodes dammini* (Acari: Ixodidae): short-term control of nymphs and larvae. J. Med. Entomol. 28: 624–629.

Schulze, T. L., G. C. Taylor, L. M. Vasvary, W. Simmons, and R. A. Jordan. 1992. Effectiveness of an aerial application of carbaryl in controlling *Ixodes dammini* (Acari: Ixodidae) adults in a high-use recreational area in New Jersey. J. Med. Entomol. 29: 544–547.

Schulze, T. L., R. A. Jordan, L. M. Vasvary, M. S. Chomsky, D. C. Shaw, M. A. Meddis, R. C. Taylor, and J. Piesman. 1994. Suppression of *Ixodes scapularis* (Acari: Ixodidae) nymphs in a large residential community. J. Med. Entomol. 31: 206–211.

Schulze, T. L., R. A. Jordan, and R. W. Hung. 1995. Suppression of subadult *Ixodes scapularis* (Acari: Ixodidae) following removal of leaf litter. J. Med. Entomol. 32: 730–733.

Schulze, T. L., R. A. Jordan, and R. W. Hung. 2000. Effects of granular carbaryl application on sympatric populations of *Ixodes scapularis* (Acari: Ixodidae) and *Amblyomma americanum* (Acari: Ixodidae) nymphs. J. Med. Entomol. 37: 121–125.

Schulze, T. L., R. A. Jordan, R. W. Hung, R. C. Taylor, D. Markowski, and M. S. Chomsky. 2001a. Efficacy of granular deltamethrin against *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) nymphs. J. Med. Entomol. 38: 344–346.

Schulze, T. L., R. A. Jordan, R. W. Hung, A. J. Krivenko, Jr., J. J. Schulze, and T. M. Jordan. 2001b. Effects of an application of granular carbaryl on nontarget forest floor arthropods. J. Econ. Entomol. 94: 123–128.

Simberloff, D., and P. Stiling. 1996. How risky is biological control? Ecology 77: 1965–1974.

Sonenshine, D. E., S. A. Allan, R.A.I. Norval, and M. J. Burridge. 1996. A self-medicating applicator for control of ticks on deer. Med. Vet. Entomol. 10: 149–154.

Spielman, A., M. L. Wilson, J. F. Levine, and J. Piesman. 1985. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. Annu. Rev. Entomol. 30: 439–460.

St. Leger, R. J. 1993. Biology and mechanisms of insect-cuticle invasion by Deuteromycete fungal pathogens, pp. 212–229. *In* N. E. Beckage, S. N. Thompson, and B. A. Federici (eds.), Parasites and pathogens of insects. Academic, San Diego, CA.

Stafford, K. C., III. 1991. Effectiveness of carbaryl applications for the control of *Ixodes dammini* nymphs in an endemic residential area. J. Med. Entomol. 28: 32–36.

Stafford, K. C., III, A. J. Denicola, and L. A. Magnarelli. 1996. Presence of *Ixodiphagus hookeri* (Hymenoptera: Encyrtidae) in two Connecticut populations of *Ixodes scapularis.* J. Med. Entomol. 33: 183–188.

Stafford, K. C., III, J. S. Ward, and L. A. Magnarelli. 1998. Impact of controlled burns on the abundance of *Ixodes scapularis* (Acari: Ixodidae). J. Med. Entomol. 35: 510–513.

Van Buskirk, J., and R. S. Ostfeld. 1995. Controlling Lyme disease by modifying the density and species composition of tick hosts. Ecol. Applic. 5: 1133–1140.

Walker, D. H., and J. S. Dumler. 1996. Emergence of ehrlichiosis as a human health problem. Emerg. Infect. Dis. 2: 18–29.

White, D. J., H. Chang, J. L. Benach, E. M. Bosler, S. C. Meldrum, R. G. Means, J. G. Debbie, G. S. Birkhead, and D. L. Morse. 1991. The geographic spread and temporal increase of the Lyme disease epidemic. J. Am. Med. Assoc. 266: 1230–1236.

Wilson, M. L., J. F. Levine, and A. Spielman. 1984. Effect of deer reduction on abundance of the deer tick (*Ixodes dammini*). Yale J. Biol. Med. 57: 697–705.

Wilson, M. L., S. R. Telford III, J. Piesman, and A. Spielman. 1988. Reduced abundance of Immature *Ixodes dammini* (Acari: Ixodidae) following elimination of deer. J. Med. Entomol. 25: 224–228.

Zhioua, E., R. A. LeBrun, H. S. Ginsberg, and A. Aeschlimann. 1995. Pathogenicity of *Steinernema carpocapsae* and *S. glaseri* (Nematoda: Steinernematidae) to *Ixodes scapularis* (Acari: Ixodidae). J. Med. Entomol. 32: 900–905.

Zhioua, E., H. S. Ginsberg, R. A. Humber, and R. A. LeBrun. 1999a. Preliminary survey for entomopathogenic fungi associated with *Ixodes scapularis* (Acari: Ixodidae) in southern New York and New England, USA. J. Med. Entomol. 36: 635–637.

Zhioua, E., M. Browning, P. W. Johnson, H. S. Ginsberg, and R. A. LeBrun. 1997. Pathogenicity of the entomopathogenic fungus *Metarhizium anisopliae* (Deuteromycetes) to *Ixodes scapularis* (Acari: Ixodidae). J. Parasitol. 83: 815–818.

Zhioua, E., K. Heyer, M. Browning, H. Ginsberg, and R. A. LeBrun. 1999b. Pathogenicity of *Bacillus thuringiensis* variety *kurstaki* to *Ixodes scapularis* (Acari: Ixodidae). J. Med. Entomol. 36: 900–902.

Zimmerman, G. 1993. The entomopathogenic fungus *Metarhizium anisopliae* and its potential as a biocontrol agent. Pestic. Sci. 37: 375–379.

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