2010

Field Applications of Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* F52 (Hypocreales: Clavicipitaceae) for the Control of *Ixodes scapularis* (Acari: Ixodidae)

Kirby C. Stafford III
Connecticut Agricultural Experiment Station, kirby.stafford@ct.gov

Sandra A. Allan
USDA-ARS, sandy.allan@ars.usda.gov

Stafford, Kirby C. III and Allan, Sandra A., "Field Applications of Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* F52 (Hypocreales: Clavicipitaceae) for the Control of *Ixodes scapularis* (Acari: Ixodidae)" (2010). *Publications from USDA-ARS / UNL Faculty*. 1064.
https://digitalcommons.unl.edu/usdaarsfacpub/1064

Field Applications of Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* F52 (Hypocreales: Clavicipitaceae) for the Control of *Ixodes scapularis* (Acari: Ixodidae)

KIRBY C. STAFFORD III[1] AND SANDRA A. ALLAN[2]

J. Med. Entomol. 47(6): 1107–1115 (2010); DOI: 10.1603/ME10019

ABSTRACT Two commercial formulations of *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin were applied to residential sites in Old Lyme, CT, for the control of nymphs of the blacklegged tick, *Ixodes scapularis*, in 1999 and 2000. The pyrethroid bifenthrin was applied to other premises for comparison with *B. bassiana*. A wood chip barrier was installed and maintained at six of the treated properties. In 1999, control of *I. scapularis* nymphs ranged from 74.5 to 83.0% on lawns without wood chips and from 88.9 to 90% on lawns with wood chip barriers. As a control check, no ticks ($n = 23$) collected at pretreatment or control sites died from *B. bassiana*, although 15 of 18 nymphs from treated lawns developed mycoses. Control of *I. scapularis* on the lawns in 2000 with the two *B. bassiana* products was lower, as follows: 38.0 and 58.7% without the barrier and 56.9 and 55.1% with the wood chip barrier. Posttreatment differences in nymphal numbers between treatments and control were significant ($P = 0.005$ and $P = 0.039$, 1999 and 2000, respectively). The bifenthrin provided 86 and 87% control each year, respectively. The application of *Metarhizium anisopliae* (Metschnikoff) Sorokin strain F52 to 9 residential sites in Westport and Weston, CT, in 2002 provided significant ($P = 0.034$; $P = 0.039$) reductions in nymphal tick abundance with 55.6 and 84.6% fewer ticks on lawn and woodland plots, respectively. These results suggest the application of entomopathogenic fungi could provide another approach for the control of *I. scapularis* nymphs in residential or similar landscapes.

KEY WORDS tick control, *Ixodes scapularis*, entomopathogen, fungi, biological control

The blacklegged tick, *Ixodes scapularis* Say, is the principal vector of *Borrelia burgdorferi* sensu stricto Johnson, Schmid, Hyde, Steigerwalt & Brenner, the causal agent of Lyme disease, in the northeastern and midwestern United States. This disease is the leading arthropod-associated disease in the United States, and the prevalence of this illness has increased dramatically in the past decade (CDC 2008). In addition to *B. burgdorferi*, the agents of human babesiosis and human granulocytic anaplasmosis are also transmitted by *I. scapularis* (Anderson et al. 1991, Magnarelli et al. 1995, Walker and Dumler 1996). Lyme disease cases are associated principally with the nymphal stage of *I. scapularis* during the summer months (Falco et al. 1999). The majority of these ticks are associated with forests, wooded suburban landscapes, and woodland edge (ecotone), but *I. scapularis* nymphs also are found on lawns and ornamental plantings, and most cases of Lyme disease are probably acquired peridomestically (Ginsberg and Ewing 1989, Maupin et al. 1991, Carroll et al. 1992, Stafford and Magnarelli 1993). Therefore, tick control efforts may be able to reduce exposure to infected ticks if widely applied in residential locations during the summer, particularly in areas of high disease incidence (Stafford and Kitron 2002).

The application of area-wide acaricides has been shown to reduce populations of *I. scapularis* in residential and woodland settings (Stafford and Kitron 2002, Ginsberg and Stafford 2005, Piesman and Eisen 2008). However, some homeowners may be reluctant to use conventional residual pesticides because of health and environmental concerns, and the organophosphates such as chlorpyrifos and diazinon are no longer registered for residential use in the United States. Alternatively, entomopathogenic fungi are reported to be major pathogens of ticks, and their use for the control of ticks appears more promising than other potential biological control agents (Samish and Rehacek 1999). Several fungi, including species of *Metarhizium, Beauvaria, Paecilomyces,* and *Lecanicillium,* have been found associated with field-collected *I. scapularis* in the northeastern United States (Zhioua et al. 1999, Tuininga et al. 2009).

Beauvaria bassiana (Balsamo-Crivelli) and *Metarhizium anisopliae* (Metschnikoff) Sorokin are two of the most important entomopathogenic fungi currently used against a wide range of arthropod, mainly insect,

---

[1] Corresponding author: Department of Entomology, Connecticut Agricultural Experiment Station, 123 Huntington Street, Box 1106, New Haven, CT 06504 (e-mail: kirby.stafford@ct.gov).

[2] Center for Medical, Agricultural, and Veterinary Entomology, United States Department of Agriculture–Agricultural Research Service, 1600/1700 SW 23rd Drive, Gainesville, FL 32608.

pests, and the most common species developed as mycopesticides (Butt et al. 2001; Zimmerman 2007a, b). Different strains of *B. bassiana* or *M. anisopliae* cause mortality in *Rhipicephalus appendiculatus, Amblyomma variegatum* (Kaaya et al. 1996), *Ixodes ricinus* (Saminakova et al. 1974), and *I. scapularis* (Zhioua et al. 1997; Benjamin et al. 2002; Hornbostel et al. 2004, 2005a, b; Kirkland et al. 2004) among other tick species, with *M. anisopliae* generally the most pathogenic species. Various strains of *M. anisopliae* are pathogenic to *I. scapularis* in the laboratory and field and could play a role in the management of tick populations (Benjamin et al. 2002, Hornbostel et al. 2005b). Some sublethal effects have also been documented against *I. scapularis* with *M. anisopliae* (Hornbostel et al. 2004). Both species of fungi occur naturally in the soil worldwide, including northeastern North America (Brownbridge et al. 1993, Bidochka et al. 1998), but natural infection rates in ticks are variable and, for *I. scapularis,* can be low (Tuininga et al. 2009). In the laboratory, two commercial formulations of *B. bassiana* with labels for ornamentals, grassland, and turf were pathogenic against nymphs of *I. scapularis* with cumulative mortalities of 74–80% by 21 d with a dose of 10^7–10^9 conidia/ml (S.A.A., unpublished data). Therefore, the purpose of this study was to examine the potential of these two commercial formulations of *B. bassiana* and a new strain of *M. anisopliae* under commercial development for the control of nymphal *I. scapularis* in residential settings endemic for *I. scapularis* and Lyme disease.

Materials and Methods

Entomopathogenic Fungi Strains. Two commercial products containing different strains of the entomopathogenic fungus *B. bassiana* were evaluated for the control of *I. scapularis* nymphs during summer of 1999 and 2000. The two *B. bassiana* products were Naturalis T&O strain ATCC 74040 (Troy BioSciences, Phoenix, AZ; now Naturalis L) and BotaniGard ES strain GHA (Mycotech, Butte, MT; now Laverlam International Corporation). The products will hereafter be referred to by the strain designation. The *B. bassiana* ATCC 74040 product contained 7.16% active ingredient based on 2.3×10^{17} viable spores/ml of product. The recommended rate for this product was 1–3 fluid ounces per 1000 feet^2, and the 3-ounce rate (95.5 ml/100 m^2) was used in this trial. The *B. bassiana* GHA product contained 11.3% active ingredient based on a weight estimate of 4.78×10^{-12} g per spore and per label, 2×10^{13} viable *B. bassiana* spores per quart (1.1×10^{10} viable spores/ml). At the time, the label for *B. bassiana* GHA did not provide a specific rate for crops and turf (other than greenhouse use). After consultation with company officials, a rate of 4.7 L/ha (two qt/acre), which equals 2.2×10^9 viable spores/100 m^2, was used. The label spore viability of *B. bassiana* was confirmed by fluorescence microscopy of a subsample of the formulated products (Firstencel et al. 1990). In brief, fungal formulations were diluted to 1×10^7 spores/ml, and 5 μl of the material was placed on a glass slide, to which fluorescein diacetate and propidium iodide were added. Spore viability was determined by fluorescing colors of the spores. Counts of germinated and ungerminated spores were made over five fields and an average calculated for each slide. Each sample was replicated on three slides.

In 2002, an oil-based laboratory formulation of the fungus *M. anisopliae* strain F52 containing 11% active ingredient was evaluated for the control of nymphal *I. scapularis* at lawn and woodland plots in Westport, Weston, and Old Lyme, CT. The material was shipped from the United Kingdom and provided by Taensa (Fairfield, CT), which subsequently became Earth BioSciences (Fairfield, CT), and was acquired by Novozymes Biologicals (Salem, VA) in 2006. Three laboratory-produced lots were used in the trials with spore viabilities of 70, 61, and 48% in the formulations (data provided by Taensa). This corresponds to 2.6×10^9 to 3.9×10^9 viable spores/ml. *M. anisopliae* F52 is currently registered with the United States Environmental Protection Agency under the labels Tick-Ex EC, Tick-Ex G, and Met52. The amount of product used in the final spray was adjusted to provide 2.5×10^{11} viable spores/100 m^2.

Treatment Sites. The *B. bassiana* spray applications were conducted at the lawn/woodland perimeter and in woodland plots at residential properties in Old Lyme, CT, in 1999 and 2000, as outlined in Table 1. In addition, both *B. bassiana* formulations were applied in 1999 and 2000 to three homes, each with a 3-m wide wood chip barrier at the lawn perimeter edge with the woods to determine whether landscape measures when combined with applications of the fungus could be more effective than the fungus alone. These barriers had been installed as part of an earlier assessment of landscape barriers, which had reduced tick activity on the lawn (S.A.A., unpublished data; Stafford 2007). Management of the edge (ecotone) by removal of leaf litter and vegetative debris at the lawn perimeter was performed at all sprayed residences in 1999 and 2000, and the wood chip barrier at the lawn edge was refurbished in mid-May 1999 and 2000. *M. anisopliae* F52 was applied to lawn/woodland perimeters and adjacent woodland plots at five of the nine residential properties in Westport and Weston or Old Lyme, CT, in 2002. Controls consisted of untreated lawns or wood plots at other residences in the same communities.

Application. The *B. bassiana* products were applied twice during the nymphal tick season with the same rates used in 1999 and 2000 (Table 1). In southern New England, nymphal *I. scapularis* become active in late May with peak activity in June, slowly declining through July, with some activity extending into August (Falco and Fish 1988, Stafford and Magnarelli 1993). The applications of *B. bassiana* were calibrated to deliver the same number of viable spores per unit area for each product each year. In 1999, the applications were made with a high-volume/high-pressure hydraulic sprayer with a capacity of 1,514 liters and an output of 32.5 L/100 m^2. In 2000, all three materials were applied with a low-volume hydraulic sprayer with a tank capacity of 189 liters and an output of ≈4.8 L/100

Table 1. Summary of treatments, site information, number of plots, range of plot size, total area treated and sampled, application rates, and dates of treatment for the application of the entomopathogenic fungi *B. bassiana* and *M. anisopliae*, and the pyrethroid bifenthrin in field trials at residential properties, 1999–2000 and 2002

Year	Treatment	Strain[a]	Location	n[b]	Site[c]	No. plots	Plot size m^2	Total area m^2	Rate of application spores per 100 m^2	Dates of application
1999	*B. bassiana*	ATCC 74040	Old Lyme	6	L	6	80–378	1,365	2.2×10^9	25 May, 29 June
	B. bassiana	ATCC 74040	Old Lyme	3	LB	3	106–154	391	2.2×10^9	25 May, 29 June
	B. bassiana	GHA	Old Lyme	7	L	7	105–283	1,318	9.9×10^{11}	2 June, 29 June
	B. bassiana	GHA	Old Lyme	3	LB	3	82–127	316	9.9×10^{11}	2 June, 29 June
	Bifenthrin	Pyrethroid	Old Lyme	5	L	5	111–201	726	14.6 ml[d]	25 May, None
	Control	–	Old Lyme	18	L	18	60–485	3,449	–	–
2000	*B. bassiana*	ATCC 74040	Old Lyme	6	L	6	80–378	1,365	2.2×10^9	5 June, 29 June
	B. bassiana	ATCC 74040	Old Lyme	3	LB	3	106–154	391	2.2×10^9	5 June, 29 June
	B. bassiana	GHA	Old Lyme	6	L	7	105–283	1,318	9.9×10^{11}	2 June, 29 June
	B. bassiana	GHA	Old Lyme	3	LB	3	82–127	316	9.9×10^{11}	2 June, 29 June
	Bifenthrin	pyrethriod	Old Lyme	5	L	5	111–201	726	14.6 ml[d]	8 June, None
	Control	–	Old Lyme	17	L	17	60–485	3,330	–	–
2002	*M. anisopliae*	F52	Westport	6	L	9	30–129	1,365	2.5×10^{11}	10 June, 3 July[e]
	M. anisopliae	F52	Westport	3	W	17	20–237	391	2.5×10^{11}	10 June, 3 July[e]
	Control	–	Westport	6	L	9	80–330	1,318	–	–
	Control	–	Westport	3	W	3	100–150	316	–	–
	M. anisopliae	F52	Old Lyme	6	L	5	25–100	1,365	2.5×10^{11}	18 June, 8 July[f]
	M. anisopliae	F52	Old Lyme	3	W	16	21–100	391	2.5×10^{11}	18 June, 8 July[f]
	Control	–	Old Lyme	6	L	7	15–50	1,318	–	–
	Control	–	Old Lyme	3	W	6	12–200	316	–	–

[a] *B. bassiana* ATCC 74040 (Naturalis TO); *B. bassiana* GHO (BotaniGard ES); *M. anisopliae* F52 (Tick-Ex EC). See text.

[b] Number of residential homesites.

[c] Application and sampling area; L = lawn, LB = lawn with wood chip barrier, W = woodland.

[d] Bifenthrin label rate of application of product per 100 m^2.

[e] Spore viabilities were 70 (10 June) and 61% (3 July), which translated to 63.7 and 76.4 ml of product/100 m^2, respectively.

[f] Spore viability for first application was 48% and was applied at a rate of 92.3 ml/100 m^2; second application consisted of a combination of available material with 61 and 48% spore viability applied at a rate of 76.4 and 92.3 ml/100 m^2, respectively.

m^2 at a pressure of 4.2 kg/cm^2 (60 psi). All treatments were made by a commercial operator contracted to make the applications under bid and supervision of K.C.S. The application rate in number of viable spores per 100 m^2 is provided in Table 1, based on manufacturer's information and testing, as previously mentioned. A commercial product containing the pyrethroid bifenthrin (Talstar, FMC Corporation, Philadelphia, PA) was used as a positive control for comparison with *B. bassiana.* The bifenthrin was a 7.9% flowable insecticide/miticide, which was labeled for application to the lawn for tick control at 7.3–14.6 ml/100 m^2. A rate of 14.6 ml/100 m^2 was used.

In the summer of 2002, two applications of the fungus *M. anisopliae* F52 were made at each treated property. Adjustments were made in the product application rate based on predetermined spore viability and volume of the specific formulation available to deliver a rate of $\approx 2.5 \times 10^{11}$ viable spores/100 m^2. Applications in Westport and Weston were made using hydraulic sprayer calibrated to deliver 8.1 L/100 m^2 finished spray at 14.1 kg/cm^2 (200 psi). Because of delays in shipment of fungal product from the United Kingdom, the treatments in Old Lyme were made slightly later in the season, with the first application on 18 June 2002. The high-volume, high-pressure hydraulic sprayer was recalibrated to deliver 8.1 L/100 m^2 at 14.1 kg/cm^2.

Tick Sampling. Ticks were sampled on a regular basis on the lawn, barrier zone, and woodlands for comparison with nonintervention (control) sites. The relative abundance of host-seeking *I. scapularis* at these homes was determined by "dragging" a 1.2-m^2 piece of flannel cloth over the vegetation at all surveyed properties. The sites sampled were the lawn edge and woodland edge, and adjacent woodland plots where *I. scapularis* predominate. For the *B. bassiana* trials, the mean lawn area sampled per home for each treatment was similar, ranging from 105.3 to 227.5 m^2 for the five treatments and control. The treated and control sites were sampled one or two times preapplication and every 2 wk afterward. There was some variation in sample dates within the biweekly period because of rain showers. Any ticks found on the drag cloth from each plot were placed in vials with a blade of grass for moisture and returned to the laboratory and held for development of mycoses as a check on possible fungal activity in the control sites or treatment sites before the fungal applications. To assess an impact on risk of an infected tick bite from the treatments, a subsample of ticks from the control sites was tested for the presence of *B. burgdorferi,* the causal agent of Lyme disease, by indirect fluorescent antibody staining of mid-gut tissues with murine monoclonal antibody (H5332) directed to outer surface protein A and fluorescein-conjugated antibodies, as previously described (Magnarelli et al. 1987).

Analysis and Percentage of Reduction. The number of ticks collected during the pretreatment period (n = 1 or 2 visits) and posttreatment period (n = 3–5 visits in June and July) for both the treatment and control was compiled as the number per site visit (i.e., premise

Table 2. Tick control treatments, number of *Ixodes scapularis* nymphs collected per site per visit and per 100 m² from lawns, pretreatment and posttreatment, 1999 and 2000, and lawns and wood plots, 2002

Year	Treatment	Strain[a]	Site[b]	No. samples (pre-, post-)[c]	Total nymphs collected	Mean no. nymphs collected			
						Per site visit		Per 100 m²	
						Pre-	Post-	Pre-	Post-
1999	*B. bassiana*	ATCC 74040	L	6, 30	24	0.7 (0.4)	0.7 (0.3)	0.4 (0.3)	0.3 (0.1)
	B. bassiana	ATCC 74040	LB	3, 15	5	0.7 (0.3)	0.2 (0.1)	0.6 (0.3)	0.2 (0.1)
	B. bassiana	GHA	L	14, 35	37	1.4 (0.3)	0.5 (0.2)	0.7 (0.1)	0.2 (0.1)
	B. bassiana	GHA	LB	6, 15	10	1.2 (0.3)	0.2 (0.1)	1.1 (0.2)	0.2 (0.1)
	Bifenthrin	Pyrethroid	L	10, 20	10	0.8 (0.5)	0.2 (0.1)	0.5 (0.3)	0.2 (0.1)
	Control	–	L	18, 90	229	0.9 (0.2)	2.3 (0.7)	0.6 (0.1)	1.2 (0.3)
2000	*B. bassiana*	ATCC 74040	L	6, 28	52	1.3 (0.8)	1.7 (0.6)	0.4 (0.2)	0.7 (0.2)
	B. bassiana	ATCC 74040	LB	3, 12	10	1.3 (0.9)	0.5 (0.5)	1.0 (0.6)	0.5 (0.3)
	B. bassiana	GHA	L	7, 28	59	2.1 (0.8)	1.6 (0.5)	1.0 (0.4)	0.8 (0.2)
	B. bassiana	GHA	LB	3, 12	23	2.0 (1.0)	1.4 (1.0)	1.8 (0.7)	1.6 (1.3)
	Bifenthrin	Pyrethroid	L	5, 20	8	0.8 (0.4)	0.2 (0.1)	0.6 (0.3)	0.1 (0.1)
	Control	–	L	17, 68	244	3.4 (1.6)	2.7 (1.3)	1.7 (0.7)	1.3 (0.5)
2002	*M. anisopliae*	F52	L	14, 36	12	0.4 (0.3)	0.2 (0.1)	0.4 (0.3)	0.2 (0.1)
	Control	–	L	33, 51	83	1.3 (0.6)	0.8 (0.2)	1.1 (0.6)	1.0 (0.2)
	M. anisopliae	F52	W	7, 20	18	1.7 (0.7)	0.2 (0.4)	3.1 (1.2)	0.2 (0.2)
	Control	–	W	19, 46	132	2.4 (0.4)	1.1 (0.3)	3.7 (1.1)	1.5 (0.4)

[a] *B. bassiana* ATCC 74040 (Naturalis TO); *B. bassiana* GHO (BotaniGard ES); *M. anisopliae* F52 (Tick-Ex EC). See text.

[b] Application and sampling area; L = lawn, LB = lawn with wood chip barrier, W = woodland.

[c] Sample size = number of homes × number of visits, pretreatment and posttreatment.

risk) and, because plot size varied, number per 100 m². Differences between treatments in the pretreatment and posttreatment periods were compared using the Kruskal-Wallis one-way analysis of variance on ranks on the number per 100 m² using SigmaPlot 11 (Systat Software, San Jose, CA). The Dunn's test for multiple comparisons between individual treatments for the analysis of variance on ranks was used because sample sizes in the treatment groups were different (Systat Software 2008). Because of the differences in dates of application for the *B. bassiana* ATCC 74040 and bifenthrin and *B. bassiana* GHA in 1999, a slightly different sample set was used for the control, there being one additional sample before the *B. bassiana* GHA applications. The Mann-Whitney rank sum test was used for comparisons between the treatment and control counts for the *Metarhizium* trials. Percentage of reduction was calculated for each property using the average number of ticks collected per 100 m² using the following modification of Abbott's formula (Henderson and Tilton 1955): percentage of reduction = 100 $(1 - X_c Y_t / X_t Y_c)$, where X_c and X_t are the pretreatment averages in the control and experimental properties, respectively, and Y_c and Y_t are the posttreatment averages in the control and experimental properties, respectively. As control sites could not be directly paired with treated sites, the average for the control sites was used in the formula for X_c and Y_c. Percentage of reduction was calculated for each treated property on ticks collected per 100 m² to produce an overall mean ± SEM reduction for each treatment (i.e., 100, 56, 79, and 80% reduction at four properties would result in an average reduction of 78.8%). A negative value for percentage of reduction at a particular property was considered a zero control. Treatment effectiveness from more of a homeowner perspective based on the cumulative mean number of ticks encountered at each property per site visit through the summer season was assessed by comparing the probability of at least one infected tick bite, assuming the ticks collected represent a potential tick bite, from the following binomial equation: $P_t = 1 - (1 - k_t)^n$, where k_t = proportion of ticks infected and n = cumulative number of ticks encountered (Ginsberg 1992).

Results

The mean number of *I. scapularis* nymphs collected at each visit and per 100 m², pretreatment and posttreatment, from the lawns for each application and the controls with *B. bassiana* in 1999 and 2000 are provided in Table 2. A total of 315 and 396 *I. scapularis* nymphs was collected from all monitored properties in 1999 and 2000, respectively. There were no significant differences in tick abundance on the lawns during the pretreatment samples between the *B. bassiana* ATCC 74040 and bifenthrin treatments and control (H = 0.537, df = 3, P = 0.911, n = 35 total samples) or *B. bassiana* GHA treatments and bifenthrin and control (H = 0.279, df = 3, P = 0.870, n = 56 total samples) in 1999.

The application of *B. bassiana* provided notable reductions of *I. scapularis* in the field during 1999. Control of *I. scapularis* nymphs ranged from 74.5 to 83.0% on lawns without wood chips and from 88.9 to 90% on lawns with wood chip barriers (Table 3). Posttreatment tick abundance was significantly different between treatments and control in 1999 (H = 16.875, df = 5, $P \leq 0.001$; and H = 11.257, df = 2, P = 0.004, *B. bassiana* ATCC 74040 and GHA subgroups, respectively). The fewest ticks were recovered with the combination of *B. bassiana* and the wood chip barrier or with bifenthrin, and only these treatments were significant by multiple comparisons using Dunn's method ($P \leq 0.05$). Although some fungal cross-con-

Table 3. Mean percentage of (SEM) reduction of *Ixodes scapularis* after treatment with bifenthrin or *Beauveria bassiana*, summer 1999 and 2000, and *Metarhizium anisopliae* F52, summer 2002

Treatment[a]	No. treated home sites	Mean % (SEM) reduction[b]		
		1999	2000	2002
B. bassiana ATCC 74040	6	83.0 (6.4)	38.0 (18.4)	–
B. bassiana ATCC 74040 + barrier	3	90.0 (5.8)	56.9 (29.7)	–
B. bassiana GHA	7	74.5 (8.6)	55.2 (16.4)	–
B. bassiana GHA + barrier	3	88.9 (6.4)	55.1 (28.6)	–
Bifenthrin	5	86.4 (8.3)	87.5 (5.8)	–
M. anisopliae F52 (lawn)	9	–	–	55.6 (14.5)
M. anisopliae F52 (woods)	5	–	–	84.6 (15.4)

[a] Data for *M. anisopliae* presented for the trials in Westport and Weston, CT.

[b] Percentage of reduction based on mean number of nymphs per 100 m^2 calculated for each property (e.g., for *B. bassiana* ATCC 74040 and barrier treatment in 1999, the reductions at the three sites were 80, 100, and 90%).

tamination of the ticks in the collection tubes from each plot was possible, the sample of nymphs collected from the lawns and held after treatment all died within 4 wk, and 83.3% (*B. bassiana* ATCC 74040, $n = 6$) and 79.2% (*B. bassiana* GHA, $n = 12$) developed mycoses with *Beauveria*, confirmed by microscopic examination by S.A.A. By contrast, none of the ticks ($n = 23$) collected before treatment or in the control developed mycosis, suggesting that reductions were the result of the fungal applications and not the natural presence of these fungi in the environment. Both *B. bassiana* and *M. anisopliae* occur naturally and have been isolated from northeastern United States soils (Bidochka et al. 1998, Tuininga et al. 2009), but entomopathogenic fungi were isolated from only 4.6% of 88 *I. scapularis* nymphs in New York (Tuininga et al. 2009). Control of *I. scapularis* on the lawns in 2000 was less, 38.0 and 55.2% without the barrier and 55.1 and 56.9% with the wood chip barrier (Table 2). There were no overall differences in tick abundance between the treatments and control during the pretreatment period in 2000 ($H = 3.510$, df = 5, $P = 0.622$). There was a significant posttreatment difference (Mann-Whitney rank sum test, $T = 28$; $n = 5{,}17$; $P = 0.023$) in nymphal abundance between the control and bifenthrin in 2000. The bifenthrin provided >85% control both years; only four to six ticks were recovered from the lawns after treatment.

The application of *M. anisopliae* to lawns and wood plots in Westport and Weston resulted in a 55.6% reduction on the lawn and 84.6% reduction in the wood plots relative to the untreated sites in the same community (Table 2). A total of 245 *I. scapularis* nymphs was collected from the control and treated properties during the pre- and posttreatment period in 2002. On the lawns, there was no significant difference in pretreatment nymphal abundance between the treated and control sites ($T = 101.0$; $n = 9{,}17$; $P = 0.231$), but there was a highly significant difference between the posttreatment collections ($T = 73.0$; $n = 9{,}17$; $P = 0.009$). Similarly, there was no difference ($T = 56.0$; $n = 5{,}16$; $P = 0.967$) during the pretreatment tick counts in the woodland plots, but there was a significant difference ($T = 27.5$; $n = 5{,}16$; $P = 0.024$) in nymphal abundance between the treatment and control during the posttreatment period. The later application of *M. anisopliae* with a lower germination rate had less of an impact in Old Lyme. Although there was a 52.6% reduction on the lawn and 60.0% reduction in the wood plots relative to the untreated sites in the same community, results were variable, with some sites showing no control. There were no significant differences between any of the treatment and control tick counts for either the lawn or wood plots ($P > 0.05$). The majority (10 of 14, 71%) of nymphal ticks recovered from the wood plots after treatment were collected during the first visit after the first application from one treated site. However, no ticks were collected from this plot during subsequent visits.

The cumulative mean number of ticks collected per site visit to each property for each treatment using *B. bassiana* or bifenthrin in 1999 and 2000 is shown in Fig. 1. In the controls, the number of nymphal ticks encountered through the summer season at the property edge was 13 and 15 for 1999 and 2000, respectively. By contrast, at the properties treated with *B. bassiana,* which also had a landscape barrier, the cumulative number of ticks for each year was only 1.7 nymphs. With an infection rate with *B. burgdorferi* of 17.9% (of 151 tested) and 16.0% (of 288 tested) for 1999 and 2000, respectively, the risk of an infected tick in the untreated controls was 92.6 and 92.8% each year, respectively. With *B. bassiana* in 1999, the risk was 49.9 and 64.1%, respectively, for the ATCC 740404 and GHA strains. The risk was similar for both fungal strains in 2000 with a risk of 76.7%. By contrast, the probabilities for the properties treated with bifenthrin for each year were 37.7 and 24.3%, respectively, of encountering an infected tick. For the properties treated with the barrier and *B. bassiana,* the probability of encountering an infected tick was 28.5 and 25.3%, respectively, for each of the 2 yr, comparable to or better than that obtained with bifenthrin. With *M. anisopliae,* tick abundance and infection with *B. burgdorferi* (6.2% of 146 tested) were low in 2002, but the application of the fungus resulted in a relative risk of an infected tick bite of 8.6% as opposed to 30.0% in the control properties (Fig. 2).

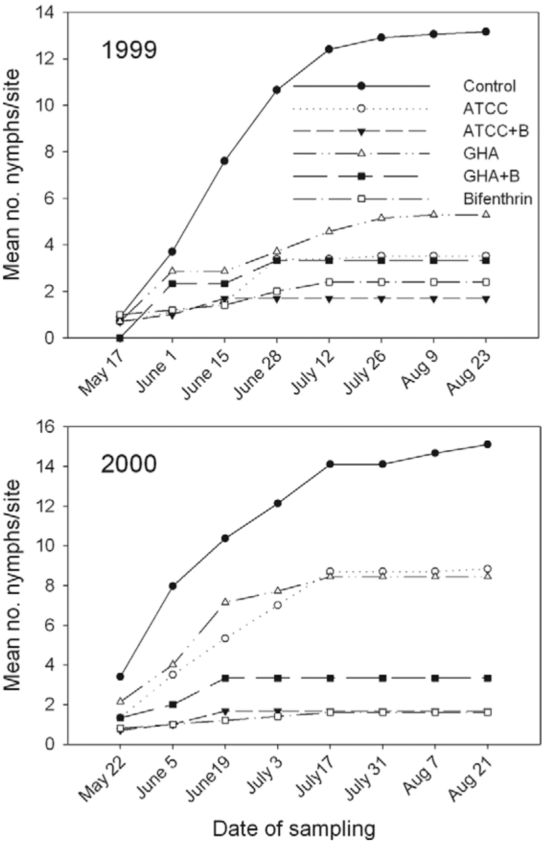

Fig. 1. Cumulative mean number of nymphal *I. scapularis* collected per site visit from lawns for the control and treatments with *B. basssiana* ATCC and GHA strains and landscape barriers (+B in legend) in Old Lyme, CT, 1999 (top) and 2000 (bottom). Legend applies to both graphs. In southern New England, nymphal tick activity appears in late May, peaks in June, and slowly declines through July, although tick activity can extend into August.

Discussion

Entomopathogenic fungi, particularly *B. bassiana* and *M. anisopliae*, have been widely used as a biopesticide for the control of a variety of insect pests, but the application of the fungi is a relatively new approach for the control of ticks. Entomopathogenic fungi have been shown to be pathogenic to *I. scapularis* in the laboratory. The current study first documents significant nymphal tick reductions after a spray application of *B. bassiana* or *M. anisopliae* in the residential landscape. These fungi generally provided moderate (>50%) to good (>75%) levels of control of host-seeking nymphal *I. scapularis.* Ticks collected from *B. bassiana*-treated areas developed mycoses from *B. bassiana* and died in contrast to the control properties or pretreatment specimens, indicating that mortality was the result of the fungal application rather than natural presence of this fungus. The efficacy of the two *Beauveria* strains was similar. Control on the lawns with the addition of a wood chip barrier was even higher than with just edge cleanup alone, suggesting that an integrated approach combining landscaping other applications could provide more effective reductions in tick activity. A landscape barrier will not affect ticks already present in the environment, but could reduce limited migration from forested edge into the lawn. Most (82%) nymphal ticks in a yard are found within 3 m of the lawn edge with woods, ecotone, or stone walls (Stafford and Magnarelli 1993). The majority of larval *I. scapularis* are found within 30 cm of the oviposition site (Stafford 1992), and the mean horizontal movement of adult *I. scapularis* is only 1.8 m (Falco and Fish 1991). Nymphal movement is unknown, but likely is much less than that of the adult tick. Landscape barrier materials can discourage tick movement (Patrican and Allan 1995), although Piesman (2006) found that of the products he evaluated, only those from Alaska yellow cedar or cellulose significantly impeded tick movement. Previous trials have shown that a wood chip barrier alone can reduce tick abundance on the lawn by an average of ≈50%, although impact is highly variable and dependent upon weathering and condition of the material (K.C.S., unpublished data).

Similarly, the oil-based *Metarhizium* F52 formulation provided good control on the lawn and woods in the trial conducted in the towns of Westport and Weston using material with relatively good spore viability (70%). Further applications with *M. anisopliae* F52 in 2007 provided a comparable level (53.2%) of tick reduction in the lawn as this study (Bharadwaj and Stafford 2010). This contrasts with the lack of an impact on tick abundance with *M. anisopliae* strain ESC1 against adults (Benjamin et al. 2002) or nymphs (Hornbostel et al. 2005b) in field plots, despite a higher rate of application reported with ESC1 ($4–6 \times 10^6$ spores/cm^2) than with F52 (2.5×10^5 spores/cm^2). Nevertheless, the laboratory mortality after 4 wk with ESC1 was 53% in 76 adult ticks collected from the treated plots versus 3% of 92 in the control group (Benjamin et al. 2002). In Old Lyme, CT, the lack of a significant difference in tick abundance observed with *M. anisopliae* F52 may be the result of the lower spore viability of the particular batch available for the first (48%) and second (between 48 and 61%) appli-

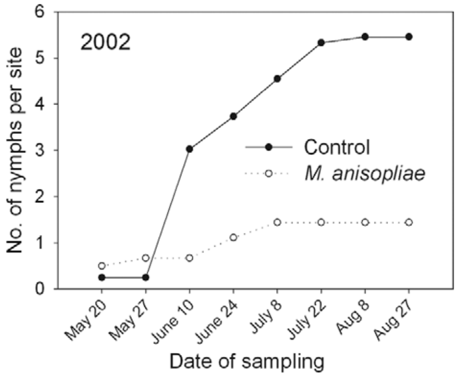

Fig. 2. Cumulative mean number of nymphal *I. scapularis* collected per site visit from lawns for the control and treatment with *M. anispoliae* F52 in Westport and Weston, 2002.

cation, despite the effort to increase the number of viable spores in the final spray, and the more limited area treated at each home the result of limited product availability. It may have taken longer for mycosis to develop, and a few unexposed ticks may have moved into the treated areas. Most (91% of 34) of the ticks were recovered in the first follow-up visit after the first application in both the lawn and wood plots, and in the wood plots most of the ticks were recovered from one site.

Low relative humidity, high temperatures, precipitation, and particularly solar ultraviolet radiation are detrimental factors to spore viability, persistence, and efficacy of these fungi in the field (Zimmerman 2007a, b). Placement of spores for optimal survival and exposure to a host-like nymphal *I. scapularis* located in the leaf litter and questing on low vegetation is not well understood. Low persistence of *B. bassiana* on exposed plant surfaces has limited utility as a foliar-applied microbial insecticide. Studies have shown that *B. bassiana* and *M. anisopliae* are less affected by ambient humidity, probably because of sufficient moisture within the microhabitat, and dry conditions during application may not be detrimental if there is adequate soil moisture. The large majority of conidia (>94%) of *B. bassiana* sprayed on the soil surface remain in the upper 5 cm of the soil (Storey and Gardner 1988) and conidia of *B. bassiana* can persist in the soil up to 2 yr (Lingg and Donaldson 1981). Conversely, rain can dislodge and disperse spores, removing it from the foliage, which may have been a factor in the *B. bassiana* trial in 2000 with above average precipitation. For example, Inyang et al. (2000) found that simulated rain to foliage reduced mortality of the beetle *Phaedon cochleariae* (F.) on oilseed rape by 42–57%, depending on the formulation. Adjustments to a formulation could improve spore performance under various environmental conditions. The addition of stickers to increase retention on foliage, however, may also limit transfer to host surface. Entomopathogenic fungi are deactivated within minutes, hours, or days when exposed to sunlight. Consequently, the use of ultraviolet protectants may enhance spore viability and reduce frequency of application, and they have been incorporated in various spore formulations (reviewed by Burges 1998; Zimmerman 2007a, b). In a separate study, examination of *B. bassiana* spores from the two products in this study on grass under field conditions indicated that ≈30% of spores were viable after 3 wk in full shade, whereas in full sun 30% of the spores were viable after only 1 wk (S.A.A., unpublished data). This suggests that field applications of commercial fungal formulations would be more effective if reapplied every 2–3 wk.

The dramatic difference in the results between 1999 and 2000 may be due, in part, to differences in application, differences in climatic conditions favoring the tick or fungi, and interannual differences in the tick population. There is little information on the optimal water volume and pressure for the application of fungal products, spore viability, and spore availability in the tick habitat. A high-volume sprayer was used in 1999, and a low-volume spray was used in 2000. Tick numbers were down both years in comparison with numbers observed previously in 1998 (Stafford et al. 1998). Weather conditions contrasted sharply between the two years, with 1999 being hot and dry (drought) and 2000 being relatively milder and wetter (Climate Impacts, Northeast Regional Climate Center, Cornell University, Ithaca, NY). In 1999, Connecticut recorded large monthly departures (2.4 and 2.1°C) from normal temperatures for June and July, respectively. June was one of the drier on record, with precipitation 12% of normal, although earlier precipitation in May was average and may have provided sufficient soil moisture (Climate Impacts, Northeast Regional Climate Center, Cornell University). By contrast, precipitation in June and July 2000 was 166 and 161% of normal, respectively, with temperatures near or below normal. Similarly, during the *Metarhizium* trials in 2002, temperatures in Connecticut were near or slightly above normal, although precipitation was above average.

Whereas fungi are not likely to be as effective for the control of ticks under as diverse conditions as synthetic chemical pesticides (generally 80 to virtually 100% control obtained) (Ginsberg and Stafford 2005), reasonably effective alternatives are needed to be able to have an option for a synthetic pesticide-free "natural" or "organic" land care or tick management program. There is a growing interest in organic land care in the northeastern United States, and standards have been established by the Northeast Organic Farming Association for the land care profession (http://www.organiclandcare.net/). Random telephone surveys of residents of several Connecticut health districts on Lyme disease knowledge, attitudes, and behaviors found acceptance of spraying a chemical pesticide was low, and generally only 22–27% reported having sprayed a chemical pesticide for tick control, although the rate increased from 22 to 44% in one district from 1999 to 2004 with educational outreach (Gould et al. 2008). A review on the safety and nontarget impacts of *B. bassiana* and *M. anisopliae* concluded that most studies found no or minimal adverse effects and that they can be considered safe, although there may be some potential for allergic reactions (Zimmerman 2007a, b). Human infections have been reported, but are rare and generally have involved immunologically compromised patients. Whereas multiple, area-wide applications could potentially affect nontarget species, the impact varies among insect species and impacts in the field are expected to be lower than that observed at some laboratory rates evaluated (Ginsberg et al. 2002). Barrier applications limited to high-risk tick habitat at the perimeters of residential properties, woodland edges, and ground-cover vegetation would further minimize nontarget affects and, in any event, the impact from a biopesticide based on nontarget studies would be expected to be substantially less than a synthetic chemical pesticide.

A number of mycoinsecticides based on *B. bassiana* or *M. anisopliae* have been commercialized and registered in various countries (Zimmerman 2007a, b), including the United States. Virulence of *M. anisopliae* varies with host and fungal strain. Whereas other strains of these or other species of fungi isolated from ticks or other hosts may prove to be more pathogenic or as effective as *M. anisopliae* F52, effective strains must be formulated, registered, and commercialized before a mycopesticide can become available to pest control professionals or homeowners. The emulsifiable concentrate formulation of *M. anisopliae* F52 used in these trials has received approval by the United States Environmental Protection Agency under the label Tick-Ex EC (Novozymes Biologicals, Salem, VA) for nonfood greenhouse and residential outdoor uses, including tick control. The product is registered in most states, and it is anticipated to be commercially available in 2011. The availability of an entomopathogenic fungus for residential tick control could provide an additional tool for an integrated approach to managing tick populations in the residential landscape.

Acknowledgments

We thank technician Heidi Stuber and numerous summer research assistants at the Connecticut Agricultural Experiment Station for their assistance with fieldwork. The assistance of the staff of the Westport Weston Health District in recruiting homes for the 2002 study is gratefully acknowledged. The *B. bassiana* was purchased through local vendors. The *M. anisopliae* strain F52 was provided by Taensa (Earth BioSciences, Fairfield, CT). This work was supported primarily, in part, by Cooperative Agreement U50/CCU419577 with the Centers for Disease Control and Prevention.

References Cited

Anderson, J. F., E. D. Mintz, J. J. Gadbaw, and L. A. Magnarelli. 1991. *Babesia microti,* human babesiosis and *Borrelia burgdorferi* in Connecticut. J. Clin. Microbiol. 29: 2779–2783.

Benjamin, M. A., E. Zhioua, and R. S. Ostfeld. 2002. Laboratory and field evalulation of the entomopathogenic fungus *Metarhizium anisopliae* (Deuteromycetes) for controlling questing adult *Ixodes scapularis* (Acari: Ixodidae). J. Med. Entomol. 39: 723–728.

Bidochka, M. J., J. E. Kasperski, and G.A.M. Wild. 1998. Occurrence of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* in soils from temperate and near-northern habitats. Can. J. Bot. 76: 1198–1204.

Brownbridge, M., R. A. Humber, B. L. Parker, and L. Skinner. 1993. Fungal entomopathogens recovered from Vermont forest soils. Mycologia 85: 358–361.

Burges, H. D. 1998. Formulation of mycoinsecticides, pp. 131–185. *In* H. D. Burges (ed.), Formulation of Microbial Biopesticides: Beneficial Microorganisms, Nematodes and Seed Treatments. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Butt, T. M., C. W. Jackson, and N. Magan [eds.]. 2001. Fungi as Biocontrol Agents: Progress, Problems and Potential. CABI Publishing, Wallingford, Oxon, United Kingdom.

Carroll, M. C., H. S. Ginsberg, K. E. Hyland, and R. Hu. 1992. Distribution of *Ixodes dammini* (Acari: Ixodidae) in residential lawns on Prudence Island, Rhode Island. J. Med. Entomol. 29: 1052–1055.

[CDC] Centers for Disease Control. 2008. Surveillance for lyme disease - United States, 1992–2006. MMWR Surveill. Summ. 57: 1–9.

Falco, R. C., and D. Fish. 1988. Ticks parasitizing humans in a Lyme disease endemic area of southern New York State. Am. J. Epidemiol. 128: 1146–1152.

Falco, R. C., and D. Fish. 1991. Horizontal movement of adult *Ixodes dammini* (Acari: Ixodidae) attracted to CO_2-baited traps. J. Med. Entomol. 28: 726–729.

Falco, R. C., D. McKenna, T. J. Daniels, R. B. Nadelman, J. Nowakowski, D. Fish, and G. P. Wormser. 1999. Temporal relation between *Ixodes scapularis* abundance and risk for Lyme disease associated with erythema migrans. Am. J. Epidemiol. 149: 771–776.

Firstencel, H., T. M. Butt, and R. I. Carruthers. 1990. A fluroescence microscopy method for determining the viability of entomopathogenic fungal spores. J. Invertebr. Pathol. 55: 258–264.

Ginsberg, H. S. 1992. Ecology and management of ticks and Lyme disease at Fire Island National Seashore and selected national parks. Scientific Mongraph NPS/NR-SUNJ/NRSM-92/20, U.S. Department of the Interior National Park Service, Washington, DC.

Ginsberg, H. S., and C. P. Ewing. 1989. Habitat distribution of *Ixodes dammini* (Acari: Ixodidae) and Lyme disease spirochetes on Fire Island, New York. J. Med. Entomol. 26: 183–189.

Ginsberg, H. S., and K. C. Stafford III. 2005. Management of ticks and tick-borne diseases, pp. 65–86. *In* J. L. Goodman, D. T. Dennis, and D. E. Sonenshine (eds.), Tickborne Diseases of Humans. ASM Press, Herndon, VA.

Ginsberg, H. S., R. A. Lebrun, K. Heyer, and E. Zhioua. 2002. Potential nontarget effects of *Metarhizium anisopliae* (Deuteromycetes) used for biological control of ticks (Acari: Ixodidae). Environ. Entomol. 31: 1191–1196.

Gould, L. H., R. S. Nelson, K. S. Griffith, E. B. Hayes, J. Piesman, P. S. Mead, and M. L. Cartter. 2008. Knowledge, attitudes, and behaviors regarding Lyme disease prevention among Connecticut residents, 1999–2004. Vector Borne Zoonotic Dis. 8: 769–776.

Henderson, C. F., and E. W. Tilton. 1955. Tests with acaricides against brown wheat mite. J. Econ. Entomol. 48: 157–161.

Hornbostel, V. L., R. S. Ostfeld, E. Zhioua, and M. A. Benjamin. 2004. Sublethal effects of *Metarhizium anisopliae* (Deuteromycetes) on engorged larval, nymphal, and adult *Ixodes scapularis* (Acari: Ixodidae). J. Med. Entomol. 41: 922–929.

Hornbostel, V. L., R. S. Ostfeld, and M. A. Benjamin. 2005a. Effectiveness of *Metarhizium anisopliae* (Deuteromycetes) against *Ixodes scapularis* (Acari: Ixodidae) engorging on *Peromyscus leucopus.* J. Vector Ecol. 30: 91–101.

Hornbostel, V. L., E. Zhioua, M. A. Benjamin, H. S. Ginsberg, and R. S. Ostfeld. 2005b. Pathogenicity of *Metarhizium anisopliae* (Deuteromycetes) and permethrin to *Ixodes scapularis* (Acari: Ixodidae) nymphs. Exp. Appl. Acarol. 35: 301–316.

Inyang, E. N., H. A. McCartney, B. Oyejola, L. Ibrahim, B. J. Pye, S. A. Archer, and T. M. Butt. 2000. Effect of formulation, application and rain on the persistence of the entomogenous fungus *Metarhizium anisopliae* on oilseed rape. Mycol. Res. 104: 653–661.

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 *Beauveria bassiana* and *Metarhizium anisopliae.* J. Invertebr. Pathol. 67: 15–20.

Kirkland, B. H., G. S. Westwood, and N. O. Keyhani. 2004. Pathogenicity of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* to Ixodidae species *Dermacentor variabilis, Rhipicephalus sanguineus,* and *Ixodes scapularis.* J. Med. Entomol. 41: 705–711.

Lingg, A. J., and M. D. Donaldson. 1981. Biotic and abiotic factors affecting stability of *Beauveria bassiana* conidia in soil. J. Invertebr. Pathol. 38: 191–200.

Magnarelli, L. A., J. F. Anderson, and D. Fish. 1987. Transovarial transmission of *Borrelia burgdorferi* in *Ixodes dammini* (Acari: Ixodidae). J. Infect. Dis. 156: 234–236.

Magnarelli, L. A., J. S. Dumler, J. F. Anderson, R. C. Johnson, and E. Fikrig. 1995. Coexistence of antibodies to tick-borne pathogens of babesiosis, ehrlichiosis, and Lyme disease in human sera. J. Clin. Microbiol. 33: 3054–3057.

Maupin, G. O., D. Fish, J. Zultowsky, E. G. Campos, and J. Piesman. 1991. Landscape ecology of Lyme disease in a residential area of Westchester County, New York. Am. J. Epidemiol. 133: 1105–1113.

Patrican, L. A., and S. A. Allan. 1995. Laboratory evaluation of desiccants and insecticidal soap applied to various substrates to control the deer tick *Ixodes scapularis.* Med. Vet. Entomol. 9: 293–299.

Piesman, J. 2006. Response of nymphal *Ixodes scapularis,* the primary tick vector of Lyme disease spirochetes in North America, to barriers derived from wood products or related home and garden items. J. Vector Ecol. 31: 412–417.

Piesman, J., and L. Eisen. 2008. Prevention of tick-borne diseases. Annu. Rev. Entomol. 53: 323–343.

Saminakova, A., S. Kalalova, M. Daniel, F. Dusbábek, E. Honzakova, and V. Cerny. 1974. Entomogenous fungi assoicated with the tick *Ixodes ricinus* (L.). Folia Parasitol. 21: 39–48.

Samish, M., and J. Rehacek. 1999. Pathogens and predators of ticks and their potential in biological control. Annu. Rev. Entomol. 44: 159–182.

Stafford, K. C., III. 1992. Oviposition and larval dispersal of *Ixodes dammini* (Acari: Ixodidae). J. Med. Entomol. 29: 129–132.

Stafford, K. C., III. 2007. Tick management handbook: an integrated guide for homeowners, pest control operators, and public health officials for the prevention of tick-associated disease. Conn. Agric. Exp. Sta. Bull. no. 1010.

Stafford, K. C., III, and U. Kitron. 2002. Environmental management for Lyme borreliosis control, pp. 301–334. *In* J. Gray (ed.), Lyme Borreliosis: Biology and Control. CABI Publishing, Oxon, United Kingdom.

Stafford, K. C., III, and L. A. Magnarelli. 1993. Spatial and temporal patterns of *Ixodes scapularis* (Acari: Ixodidae) in southcentral Connecticut. J. Med. Entomol. 30: 762–771.

Stafford, K. C., III, M. L. Cartter, L. A. Magnarelli, S. Ertel, and P. A. Mshar. 1998. Temporal correlations between tick abundance and prevalence of ticks infested with *Borrelia burgdorferi* and increasing incidence of Lyme disease. J. Clin. Microbiol. 36: 1240–1244.

Storey, G. K., and W. A. Gardner. 1988. Movement of an aqueous spray of *Beauveria bassiana* into the profile of four Georgia soils. Environ. Entomol. 17: 135–139.

Systat Software. 2008. SigmaPlot 11 user's guide part 2-statistics. Systat Software, San Jose, CA.

Tuininga, A. R., J. L. Miller, S. U. Morath, T. J. Daniels, R. C. Falco, M. Marchese, S. Sahabi, D. Rosa, and K. C. Stafford III. 2009. Isolation of entomopathogenic fungi from soils and *Ixodes scapularis* (Acari: Ixodidae) ticks: prevalence and methods. J. Med. Entomol. 46: 557–565.

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

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

Zhioua, E., H. S. Ginsberg, R. A. Humber, and R. A. Lebrun. 1999. 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.

Zimmerman, G. 2007a. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii.* Biocontrol Sci. Tech. 17: 553–596.

Zimmerman, G. 2007b. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae.* Biocontrol Sci. Tech. 17: 879–920.

Received 29 January 2010; accepted 22 July 2010.