Experimental and Applied Acarology (2005) 35: 301–316
DOI 10.1007/s10493-004-5437-z

Pathogenicity of *Metarhizium anisopliae* (Deuteromycetes) and permethrin to *Ixodes scapularis* (Acari: Ixodidae) nymphs

V. L. HORNBOSTEL[1], ELYES ZHIOUA[2,3], MICHAEL A. BENJAMIN[1], HOWARD S. GINSBERG[4] and RICHARD S. OSTFELD[1,*]

[1]*Institute of Ecosystem Studies, Box AB (Route 44A), Millbrook, NY 12545-0129, USA;* [2]*Tick Research Laboratory, Fisheries Animal and Veterinary Sciences Department, 127 Woodward Hall, University of Rhode Island, Kingston, RI 02881, USA;* [3]*Institut Pasteur, Laboratoire Ecologie des Systèmes Vectoriels, 13 Place Pasteur, BP 74 1002 Tunis-Belvédère, Tunisia;* [4]*USGS Patuxent Wildlife Research Center, Woodward-PLS, University of Rhode Island, Kingston, RI 02881, USA;* *Author for correspondence (e-mail: rostfeld@ecostudies.org; phone: +1-845-677-7600, ext. 136; fax: +1-845-677-5976)*

Received 3 May 2004; accepted in revised form 15 October 2004

Key words: Acaricide, Biological control, *Ixodes scapularis*, Lyme disease, *Metarhizium anisopliae*, Tick control

Abstract. Effectiveness of the entomopathogenic fungus *Metarhizium anisopliae*, for controlling nymphal *Ixodes scapularis*, was tested in laboratory and field trials. In the laboratory, *M. anisopliae* (Metschnikoff) Sorokin strain ESC1 was moderately pathogenic, with an LC_{50} of 10^7 spores/ml and induced 70% mortality at 10^9 spores/ml. In a field study, however, 10^9 spores/ml *M. anisopliae* did not effectively control questing *I. scapularis* nymphs, and significant differences were not detected in pre- and post-treatment densities. For nymphs collected and returned to the laboratory for observation, mortality was low in treatment groups, ranging from 20 to 36%. To assess whether a chemical acaricide would synergistically enhance pathogenicity of the fungus, we challenged unfed nymphal *I. scapularis* with combinations of *M. anisopliae* and permethrin, a relatively safe pyrethroid acaricide, in two separate bioassays. Significant interactions between *M. anisopliae* and permethrin were not observed, supporting neither synergism nor antagonism.

Introduction

In North America *Ixodes scapularis* SAY is the principal vector of *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwald & Brenner (Burgdorfer et al. 1982; Johnson et al. 1984), the bacterium causing Lyme disease, the protozoan *Babesia microti* Franca (Spielman et al. 1985), and the bacterium *Anaplasma phagocytophilum* (Pancholi et al. 1995; Walker and Dumler 1996; Dumler et al. 2001), which cause babesiosis and human granulocytic ehrlichiosis, respectively. The Centers for Disease Control and Prevention (CDC) reported 23,763 Lyme disease cases nationwide in 2002, continuing a recent trend of increased incidence (CDC 2003). Because risk of human exposure to these diseases is related to the local abundance of ticks, reducing *I. scapularis* density is critical

to integrated Lyme disease management (White et al. 1991; Daniels and Fish 1995; Ostfeld 1997). Finding the most effective *I. scapularis* control that imposes the least environmental and economic costs is an ongoing challenge. Chemical application, while costly to the environment, has been the most effective and widely used control method (Stafford 1997) for *I. scapularis* and other ixodid ticks. Biological control agents, such as the entomopathogenic fungus *Metarhizium anisopliae*, appear safer but are generally less effective than chemicals. Combining low doses of chemical acaricides to enhance pathogenicity of entomopathogenic fungi against *I. scapularis* could be an effective integration of both control methods.

Chemical application continues to be the cornerstone of *I. scapularis* and ixodid control worldwide, but can induce ixodid resistance (e.g., Nolan 1990; Beugnet and Chardonnet 1995; Fernandes 2001; Miller et al. 2001; Soderlund and Knipple 2003) and negatively affect non-target organisms (Schulze et al. 2001b). In field studies, broadcast and barrier synthetic chemical applications using organophosphates, carbamates, or pyrethroids have effectively controlled *I. scapularis* adults (Schulze et al. 1987, 1992; Patrican and Allan 1995), nymphs (Schulze et al. 1991, 1994, 2000, 2001a; Stafford 1991; Solberg et al. 1992; Curran et al. 1993; Allan and Patrican 1995; Patrican and Allan 1995), and larvae (Schulze et al. 1991; Allan and Patrican 1995). Other control methods, such as host exclusion or reduction (Stafford 1993; Daniels and Fish 1995) and environmental modifications (Schulze et al. 1995; Stafford et al. 1998) vary in their effectiveness.

Biological control, however, is a promising option for reducing *I. scapularis* density. Entomopathogenic fungi, especially *Metarhizium anisopliae* and *Beauveria bassiana*, have proven virulent to all stages of ixodid ticks (Gindin et al. 2002). *M. anisopliae* ESC1 induced high mortality in engorged *I. scapularis* larvae, engorged females (Zhioua et al. 1997), and unfed adults in the laboratory (Benjamin et al. 2002), and in questing adults under field conditions (Benjamin et al. 2002). Various strains of *M. anisopliae* have induced significant mortality in all stages, fed and unfed, of other ixodid ticks in the laboratory (e.g., Mwangi et al. 1995; Kaaya et al. 1996; Correia et al. 1998; Frazzon et al. 2000; Kaaya 2000; Onofre et al. 2001; Paiao et al. 2001; Reis et al. 2001; Samish et al. 2001; Gindin et al. 2002) and under field conditions (Kaaya et al. 1996; Kaaya 2000; Kaaya and Hassan 2000). Entomopathogenic fungi, which are natural pathogens of ixodid ticks, are expected to have lower toxicity and weaker environmental impacts (Croft 1990; Zimmermann 1993; Lacey et al. 2001; Ginsberg et al. 2002; Johnson et al. 2002) than chemical controls.

Combinations of low doses of chemical acaricides with entomopathogenic fungi may also provide safe and effective *I. scapularis* control while reducing volumes of chemical pesticides used. Researchers have combined pyrethroids, organophosphates, carbamates, or nicotinoids with *M. anisopliae* isolates, finding synergistic (Kaakeh et al. 1997; Quintela and McCoy 1997), antagonistic (Mohamed et al. 1987), additive (Mohamed et al. 1987; Kaaya et al. 1996; Kaaya and Hassan 2000), and mixed (Li and Holdom 1994;

Pachamuthu and Kamble 2000) interactions in bioassays and in growth, sporulation, and conidial germination experiments. *M. anisopliae* and chemical acaricide combinations have not previously been tested against *I. scapularis*.

In this study, we examined the pathogenicity of *M. anisopliae* Sorokin strain ESC1 against *I. scapularis* nymphs, since targeting the *I. scapularis* nymphal stage could result in immediate reduction in risk of exposure to tick-borne diseases. First we tested *M. anisopliae* against unfed nymphs under laboratory conditions. We then applied *M. anisopliae* under field conditions to target questing nymphs. Finally, we treated unfed nymphal *I. scapularis* with combinations of permethrin and *M. anisopliae* to assess any interactive effects on *I. scapularis* mortality.

Materials and methods

Laboratory experiments to determine pathogenicity of M. anisopliae *to unfed nymphal* I. scapularis

Questing nymphal *I. scapularis* were collected from forest plots at the Institute of Ecosystem Studies (IES) in Millbrook, New York (Dutchess County) and were kept under humid conditions at 2 °C until a few hours before the experiment, at which time they were placed in an incubator at 25 °C. Unfed nymphs were treated with an *M. anisopliae* (strain ESC1; Bio-Blast Biological Termiticide™) and deionized (DI) water suspension at concentrations of 10^6 to 10^9 spores/ml (the spore concentration was confirmed using a hemacytometer). We conducted five trials, with 10 nymphs per trial, for each of four *M. anisopliae* concentrations and for the DI water control (50 ticks at each concentration, 250 ticks total). For each trial, we placed 10 nymphs on filter paper in a 10 by 1.5 cm Petri dish and sprayed the entire area with 2.8 ml of spore suspension (or DI water for control) using a hand sprayer. Immediately after treatment, ticks were placed in clean (18 ml) snap-cap specimen vials, with 10 ticks per vial. Each vial was covered tightly with mesh fabric. Ticks in vials were placed in a humid chamber (Nalgene desiccator containing DI water) and incubated for 4 weeks at optimal humidity (±90% relative humidity) and temperature (23 °C) for both *I. scapularis* and *M. anisopliae* (Daoust and Roberts 1983; Daniels et al. 1989). Nymphs were examined weekly for mortality and signs of fungal mycelia, and were considered killed by fungus when mycelia were observed on tick cadavers.

To determine whether there were significant differences in numbers of ticks surviving at different fungal spore concentrations, we used the Kruskal–Wallis test (Wilkinson et al. 1992, SYSTAT for Macintosh, version 5.2, subprogram Npar). The LC_{50} was determined after 4 weeks using the inhibition concentration approach (Lewis et al. 1994).

Field trials testing pathogenicity of M. anisopliae *on questing nymphs*

Field trials at two IES sites located about 12 km apart, Route 44A and Tompkins Farm, were performed between 10 and 29 July 2001, during the peak activity period for nymphal *I. scapularis* at the study sites (Ostfeld et al. 1996). The Route 44A site features level terrain with a maple-dominated canopy and Japanese barberry (*Berberis thunbergii*) understory, while the Tompkins Farm site has a maple-dominated canopy with no shrub layer. High *I. scapularis* densities have been recorded at adjacent study sites since at least 1985 (Goodwin et al. 2001; Ostfeld et al. 2001). We established an experimental grid of six plots at each site, with each plot measuring 15 × 15 m, with a minimum of 10 m between adjacent plots; three plots at each site were randomly designated treatment and three control. Drag sampling using a 1 m^2 white corduroy drag cloth was performed to obtain baseline tick counts at the Route 44A and Tompkins Farm sites on 12 and 15 July 2001, respectively. At the Tompkins Farm site, we slowly dragged the cloth along 15 adjacent, parallel transects in each plot. Because of an extremely high tick density at the Route 44A site, we dragged along only eight parallel transects. At the end of each transect, all nymphs attached to the cloth and our clothing were counted and released on the leaf litter along the same transect. Where obstructions prevented dragging, transects were limited to 10 m. The drag cloth and clothing were carefully examined before leaving and entering plots to avoid transporting ticks between plots.

On 20 July 2001, between 0900 and 1700 h, treatment plots were sprayed with a 10^9 spores/ml Bio-Blast + DI water suspension, and control plots were treated with DI water alone. Using an 8 l lawn and garden sprayer, we passed through each plot approximately five times to mist the forest floor and overlying vegetation in 2-m wide swaths, concentrating the spray at the leaf litter level. The suspension was applied at a rate of 1.0–1.5 l per 100 m^2 . Post-treatment drag sampling (to determine tick numbers present after treatment compared with before treatment, for percentage control) and tick collection (to compare tick mortality between treatment and control sites and mortality from *M. anisopliae* infection) were performed at both sites 1, 3, and 4 weeks after treatment (27 July, 8/9 August, 15 August 2001). Drag sampling was performed on all plots in each site. Collected ticks were counted, placed in 18 ml snap-cap vials (all ticks from a given plot were placed in the same vial), incubated in humid chambers (as described previously), and monitored for 4 weeks. Spraying and drag sampling were performed only during non-rainy days.

To determine whether 10^9 spores/ml *M. anisopliae* applied in the field plots had an effect on the number of nymphs post-treatment compared with pre-treatment, the percentage control of nymphs in field plots was calculated according to Mount (1981): % control = 100 − ([{mean in treated area after treatment/mean before}/{mean in untreated area after treatment/mean before}] × 100). Changes through time in nymphal tick density per m^2 between

control and treatment plots were compared using ANOVA (Wilkinson et al. 1992, SYSTAT, subprogram MGLH, General Linear Model). Differences in mortality in ticks brought back to the laboratory for incubation were tested by comparing the number alive and the number dead from treatment and control plots using the Yates-corrected chi-square test.

Laboratory trials using permethrin and M. anisopliae *on unfed nymphal* I. scapularis

For all of the following experiments, we used unfed *I. scapularis* nymphs collected, stored, and prepared for treatment as for the laboratory experiments with *M. anisopliae* alone (described above). The *M. anisopliae* source and method of spore suspension preparation are the same as above for all of the following experiments. In these bioassays, permethrin (BONIDE® Carpenter Ant Control Concentrate, 2.5% active ingredient) was serially diluted with DI water to obtain desired concentrations.

Preliminary bioassays with permethrin

On 12 August 2002, unfed nymphal *I. scapularis* were treated with five different concentrations of permethrin (0, 0.25, 2.5, 25, 250 ppm) to determine appropriate sublethal permethrin concentrations for synergy assays with *M. anisopliae*. Each concentration was tested on four groups of 5 ticks per trial (100 ticks total). For each trial, five nymphs were placed on Whatman #1 filter paper in a 10 by 1.5 cm Petri dish, and 5 μl of DI water followed by 1 μl of permethrin was pipetted onto the dorsal side of each tick. Each nymph was then placed in an 11.1 ml snap-cap vial (covered with mesh fabric and cap with hole) and incubated in a humid chamber (Nalgene desiccator containing 150 ml of DI water, >90% RH) at 25 °C with 15:9 h light/dark. Ticks were examined daily for 3 days, at which time the preliminary assay was completed; based on the results, concentrations between 0 and 0.25 ppm permethrin were chosen for use in synergy assays.

Metarhizium anisopliae*–permethrin synergy bioassay 1*

On 29 August 2002, unfed nymphal *I. scapularis* were treated with 12 total combinations of *M. anisopliae* spore solution (at three concentrations: 0, 10^7, 10^8 spores/ml) and permethrin (at four concentrations: 0, 0.025, 0.05, 0.25 ppm) including controls (DI water). We assayed all twelve (3 fungus × 4 permethrin) combinations for synergy. Eight of the combinations were tested on four groups of five ticks per trial (20 ticks by 8 combinations = 160 ticks), while four combinations (0 + 0.0, 10^7 + 0.0, 10^7 + 0.05, and 10^8 + 0.25

spores/ml *M. anisopliae* + ppm permethrin) were each tested on nine groups of five ticks per trial (45 ticks by 4 combinations = 180 ticks; 340 total ticks tested). For each trial, nymphs were treated as described in the preliminary assay above, except 5 μl of fungal suspension followed by 1 μl of permethrin was pipetted onto the dorsal side of each tick. For the control, 5 μl of DI water plus 1 μl of DI water was used; for *M. anisopliae* controls, 5 μl of fungal suspension plus 1 μl of DI water was used; and for the permethrin control, 5 μl of DI water plus 1 l of permethrin was used. As described in the preliminary bioassay above, nymphs were individually placed in vials and incubated. Ticks were examined twice weekly for 4 weeks. Mortality and appearance of fungal mycelia were recorded.

Metarhizium anisopliae–*permethrin synergy bioassay 2*

On 1 July 2003, another bioassay was performed, as above, but with 16 different combinations of *M. anisopliae* (four concentrations: 0, 10^7, 10^8, 10^9 spores/ml) and permethrin (four concentrations: 0, 0.1, 0.25, 1.0 ppm), with each combination tested on five groups of five ticks each (16 combinations, 25 ticks per combination = 400 ticks total). Methods were the same as for the previous assay, except the volume of each chemical was doubled and the order of permethrin and *M. anisopliae* applications was reversed, such that 2 μl of permethrin was applied first, with 10 μl of *M. anisopliae* solution applied 1 min later to each tick. Controls were as for the previous assay, but with doubled volumes.

For *M. anisopliae* and permethrin bioassays, the average proportion mortality in each group was transformed according to Freeman and Tukey (1950 in Zar 1999) and analyzed for interaction using a two-way ANOVA (SYSTAT version 10).

Results

Pathogenicity of M. anisopliae *to unfed nymphal* I. scapularis *in laboratory and field trials*

Metarhizium anisopliae was pathogenic to unfed nymphal *I. scapularis* in the laboratory. Survival of unfed nymphs exposed to different concentrations of *M. anisopliae* spores varied significantly (Kruskal–Wallis test statistic = 20.007, df = 4, $p = 0.0005$, Figure 1). At the highest concentration tested, 10^9 spores/ml, 70% nymphal mortality was observed after 4 weeks. The LC_{50} (concentration to kill 50%) was 10^7 spores/ml *M. anisopliae*.

In the field tests to determine the effectiveness of *M. anisopliae* on questing *I. scapularis* nymphs, the percentage control (formula of Mount 1981) in treatment vs. control plots was modest (Table 1). Nymphs per m^2 in treated

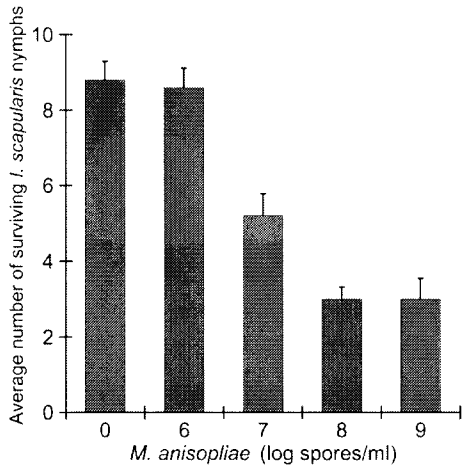

Figure 1. Mean number (+SEM) of nymphal *I. scapularis* surviving 4 weeks after fungal (*Metarhizium anisopliae*) application as a function of spore concentration. Survival decreased significantly with increasing concentration ($p = 0.0005$) and the $LC_{50} = 10^7$ spores/ml.

Table 1. Percentage control (based on formula of Mount 1981) of nymphal *I. scapularis* in field trials using the entomopathogenic fungus *Metarhizium anisopliae* at 10^9 spores/ml.

Weeks after treatment	Percentage control at:	
	Site Route 44A	Site Tompkins Farm
1	6.3	20.4
3	19.9	35.7
4	12.2	26.5

Numbers indicate percentage by which nymphal densities were reduced in fungus-treated vs. control plots.

vs. control plots did not change significantly from pre- to post-treatment, and there was no interaction of treatment by date (interaction of treatment by date: Route 44A site, $F = 0.497$, df = 3, 184, $p = 0.685$; Tompkins Farm site, $F = 1.877$, df = 3, 352, $p = 0.133$; Figure 2).

The percentage mortality of nymphs collected from field sites and incubated in the laboratory for 4 weeks demonstrated a low to moderate effect of *M. anisopliae* treatment on questing nymphs (Table 2). For the Route 44A site, nymphal mortality was significantly greater in treatment vs. control plots for nymphs collected 3 and 4 weeks, but not 1 week, after treatment (Table 2). For the Tompkins Farm site, nymphal mortality was significantly greater on treatment plots after 1 week, but not after 3 or 4 weeks (Table 2). All nymphs collected from treated plots at both field sites that died in the lab were colonized by *M. anisopliae* mycelia, while only one of those collected from control sites that subsequently died was colonized by mycelia.

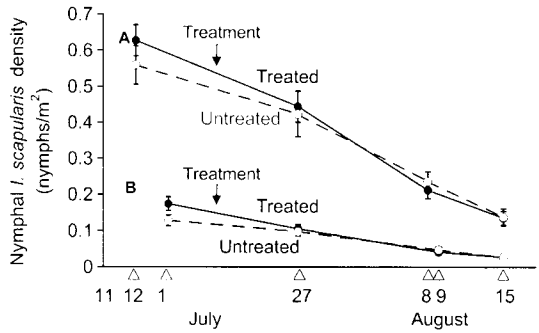

Figure 2. Densities (± SEM) of nymphal *I. scapularis* as a function of date of sampling from leaf litter before and after fungal (*Metarhizium anisopliae* at 10^9 spores/ml) treatment. The two field sites at the Institute of Ecosystem Studies in Millbrook, New York, USA are: Route 44A site (A) and Tompkins Farm site (B). 'Treatment' arrow indicates date of field application of fungus ('treated') or water ('untreated'). No significant effect of field application of fungus on population densities of nymphal ticks was observed (see Results).

Table 2. Percentage mortality of nymphal *I. scapularis* brought back to laboratory following field spraying with *Metarhizium anisopliae* solution (10^9 spores/ml).

Weeks after treatment	Site Route 44A		p^a	Site Tompkins Farm		p^a
	Treated	Untreated		Treated	Untreated	
	Control (%)			Control (%)		
1	17.1	9.9	0.084	30.9	14.8	0.050
2	36.1	11.3	0.0006	34.6	26.7	0.723
3	20.9	4.3	0.037	23.5	17.6	1.000

Numbers are percentage control (formula of Mount 1981) in fungus-treated and control (untreated) field plots.
[a]Yates-corrected chi-square tests, df = 1.

Laboratory trials testing synergism between permethrin and M. anisopliae *on unfed nymphal* I. scapularis

In the 2002 3-day preliminary bioassay with permethrin, average mortalities (proportion of ticks dying) were 0, 0.4, 0.85, 0.85, and 1.0 for 0, 0.25, 2.5, 25, and 250 ppm permethrin, respectively. Because we wanted to choose the lowest effective permethrin concentrations that would not dominate the interaction in synergy assays, we chose to use permethrin concentrations between 0 and 0.25 ppm, which induced less than 50% mortality. For the 2002 *M. anisopliae*–permethrin bioassay, average nymphal mortality 23 days post-treatment for each combination of permethrin and *M. anisopliae* tended to increase with increasing concentration of both agents (Figure 3). While the

main effect of permethrin or *M. anisopliae* alone was significant, we observed no significant interaction overall between permethrin and *M. anisopliae* (permethrin alone, $F = 3.930$, df $= 3$, $p = 0.013$; *M. anisopliae* alone, $F = 13.505$, df $= 2$, $p < 0.0001$; permethrin–*M. anisopliae* interaction, $F = 1.896$, df $= 6$, $p = 0.098$). In the 2003 *M. anisopliae*–permethrin bioassay, data from all trials with 10^8 spores/ml *M. anisopliae* were removed from analysis due to desiccation of nymphs in the 10^8 *M. anisopliae* control. Again, average nymphal mortality for the 2003 assay 23 days post-treatment generally increased with increasing concentrations of both agents (Figure 4). Analysis of the 2003 assay results again showed significant effects of permethrin and *M. anisopliae* alone, but no significant interaction overall between permethrin and *M. anisopliae* (permethrin alone, $F = 7.394$, df $= 3$, $p = 0.0003$; *M. anisopliae* alone, $F = 5.301$, df $= 2$, $p = 0.008$; permethrin–*M. anisopliae* interaction, $F = 1.506$, df $= 6$, $p = 0.197$).

Discussion

We targeted unfed and questing nymphal *I. scapularis*, because controlling their populations during their peak activity period can reduce the immediate risk of exposure to Lyme disease and potentially reduce subsequent adult *I. scapularis* density. Under laboratory conditions, *M. anisopliae* strain ESC1 was moderately pathogenic to unfed nymphal *I. scapularis*, with the same LC_{50} (10^7 spores/ml) after 4 weeks as for unfed adult *I. scapularis* (Benjamin et al. 2002) under the same experimental conditions. Below a concentration

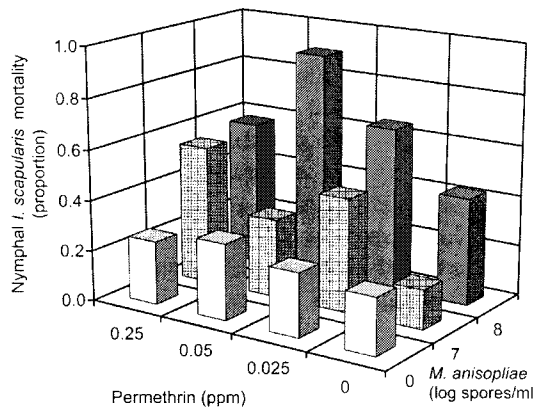

Figure 3. Proportion of *I. scapularis* nymphs dying during the 2002 experiment using combined permethrin–*M. anisopliae* application. Each bar represents a unique combination of dosages of permethrin and fungus. Although increasing dosages of both agents caused significant increases in tick mortality, the interaction term was not significant (see Results).

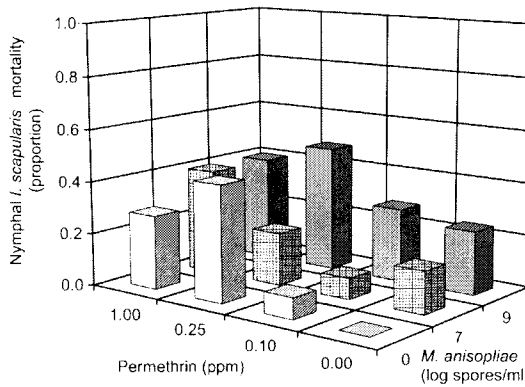

Figure 4. Proportion of *I. scapularis* nymphs dying during the 2003 experiment using combined permethrin–*M. anisopliae* application. Each bar represents a unique combination of dosages of permethrin and fungus. Although increasing dosages of both agents caused significant increases in tick mortality, the interaction term was not significant (see Results).

of 10^6 spores/ml, *M. anisopliae* did not cause significant mortality to either unfed nymphs (present results) or adults (Benjamin et al. 2002). At 10^8 and 10^9 spores/ml *M. anisopliae*, however, nymphal mortality was only approximately 70% at both concentrations, while adult mortality was about 75% and nearly 100%, respectively (Benjamin et al. 2002). This suggests that adult *I. scapularis* are more susceptible to *M. anisopliae* ESC1 than nymphs.

Consistent with our observations for *I. scapularis*, Reis et al. (2001) reported that the LC_{50} of *M. anisopliae* (isolates 959, 319, and E9) was higher (5×10^{10} to almost 3×10^{11} spores/ml) for nymphal *Amblyomma cajennense* than for adults ($1.4–4.4 \times 10^8$); they also found that at 10^8 spores/ml, *M. anisopliae* induced 63–76% and 32–45% mortality in unfed *A. cajennense* adults and nymphs, respectively. Samish et al. (2001), however, reported that 10^7 spores/ml *M. anisopliae* (isolates 7, 43, and 108) induced 40–100% mortality only 7 days post-infection against unfed nymphal *Rhipicephalus sanguineus*. Furthermore, Samish et al. (2001) found that *M. anisopliae* isolates were less pathogenic to unfed *R. sanguineus* adults than to nymphs.

In field trials, *M. anisopliae* applied at 10^9 spores/ml did not effectively control questing nymphal *I. scapularis*. This could have resulted, in part, from the aqueous formulation used in these trials. Kaaya and Hassan (2000) found that mortality of unfed nymphal *R. appendiculatus* was greater when treated with oil-based than with aqueous formulations. For *I. scapularis* nymphs collected from the field and incubated in the laboratory, significant differences in mortality between treatment and control were found in only a few instances (see Table 2). Mortalities were low, on average, ranging from 20 to 36%. Under similar experimental conditions, 53% mortality (vs. only 3% in the control group) was achieved with *M. anisopliae* (4×10^9 spores/ml) against questing adult *I. scapularis* collected 1 week post-treatment and incubated

(Benjamin et al. 2002). Nymphal *I. scapularis* collected 1 week post-treatment averaged 24% mortality. Interestingly, questing adult and nymphal mortalities were similarly lower (47 and 46%, respectively) under field conditions than in the laboratory. Factors that may reduce *M. anisopliae* field effectiveness include weather events, heat, and ultraviolet radiation (Alves et al. 1998).

Metarhizium anisopliae *and permethrin bioassays*

We studied the possible synergy between *M. anisopliae* ESC1 and sublethal doses of permethrin, a synthetic pyrethroid chemical, to enhance *M. anisopliae* pathogenicity against *I. scapularis*. The synthetic pyrethroids as a group are highly effective acaricides, and permethrin has relatively low mammalian toxicity, is environmentally safer than older acaricides, such as organophosphates and carbamates (Curran et al. 1993; Schulze et al. 2001a), and for ticks not resistant, can be effective at much lower doses than other acaricides (Curran et al. 1993; Schulze et al. 2001a; Elliott 1977 in Croft 1990). Negative impacts on microorganisms, aquatic invertebrates, pollinators, fish, birds, and mammals can, however, be significant (Smith and Stratton 1986; Gassner et al. 1997). In addition, tick resistance to pyrethroid chemicals has already developed (e.g., Nolan 1990; Beugnet and Chardonnet 1995; Fernandes 2001; Miller et al. 2001; Soderlund and Knipple 2003). Finding synergy between *M. anisopliae* and permethrin could allow for reductions in pyrethroid concentrations and volumes used in ixodid control, thereby curbing pyrethroid resistance as well as other negative environmental impacts.

In the synergy bioassays, we expected that permethrin would stress unfed *I. scapularis* nymphs, enabling the fungus to penetrate more easily and to act more effectively. While the results from both bioassays did not reveal significant interactions between permethrin and *M. anisopliae* at various concentrations, the relatively low p value (0.098) for the interaction in the first assay indicates the potential for synergy between the two chemicals. From the first bioassay, the combination inducing the highest mortality (approximately 90%) was 10^8 spores/ml *M. anisopliae* and 0.05 ppm permethrin. Mortality was reduced by over 30%, however, when permethrin was increased to 0.25 ppm. In contrast, in the second bioassay, mortality decreased substantially from 44% for 0.25 ppm permethrin alone to 20% when 10^7 spores/ml *M. anisopliae* was added with 0.25 ppm permethrin. While results varied in both bioassays, in most cases mortality was greater with application of both permethrin and *M. anisopliae* than with *M. anisopliae* alone (see Figures 3 and 4).

Our results are in general agreement with findings of other researchers, indicating that when combined with *M. anisopliae*, most pyrethroids are not inhibitory and are additive in some cases. Using a different pyrethroid, cyfluthrin (0.05–40 ppm), combined with 4×10^8 spores/ml *M. anisopliae* ESC1 in bioassays against the German cockroach (*Blattella germanica* L.), Pachamuthu and Kamble (2000) found additive effects, reporting that mortality caused by

cyfluthrin plus *M. anisopliae* was significantly greater than for cyfluthrin alone. The combination also decreased mortality times and overall pesticide volume used. Assessing 10,000-fold higher pesticide concentrations than in our bioassays, Mohamed et al. (1987) found that 5000–25,000 ppm permethrin was not inhibitory to *M. anisopliae* (E9) sporulation, mycelial growth, or conidial germination. Clark et al. (1982) reported that permethrin did not inhibit mycelial growth of *B. bassiana*, an entomopathogenic fungus similar to *M. anisopliae*. Lecuona et al. (2001) found that deltamethrin did not inhibit any *B. bassiana* strains tested in the laboratory, although other pyrethroids, unless used at very low concentrations, did inhibit growth. In one of the few studies aimed at testing interactions of chemicals and fungi specifically for the purposes of ixodid control, Kaaya et al. (1996) and Kaaya and Hassan (2000) found that *B. bassiana* and *M. anisopliae* incubated in organophosphate acaricides (commonly used in Africa against ticks) for up to 120 h retained normal growth and morphology. On the other hand, Li and Holdom (1994) found that *M. anisopliae* growth and sporulation were generally not inhibited by two carbamate insecticides, while organophosphates had variable effects, sometimes inhibitory.

Researchers have also reported synergism between various insecticides and entomopathogenic fungi. Lecuona et al. (2001) indicated that some concentrations of deltamethrin and low concentrations of beta-cypermethrin actually increased *B. bassiana* conidial germination; Anderson and Roberts (1983) observed similar interactions. Hiromori and Nishigaki (2001) found synergism between *M. anisopliae* and fenitrothion (an organophosphate) or teflubenzuron (insect growth regulator) when tested against scarab beetle larvae (*Anomola cuprea*); they attributed the synergy to weakening of the immune system by insecticidal stress, facilitating infection of *M. anisopliae* to the larvae. Imidacloprid (a nicotinoid pesticide) and *M. anisopliae* combinations, tested against larvae of the citrus root weevil (*Diaprepes abbreviatus* L.), were synergistic (Quintela and McCoy 1997). Alternatively, Anderson and Roberts (1983) found that permethrin at 959 ppm caused about 20% inhibition of *B. bassiana* (various isolates) spore germination; they observed that for some insecticides, the insecticide formulation, not the active ingredient, caused fungal inhibition, but that in this case the active ingredient in permethrin caused inhibition.

Our findings, combined with those of other researchers, indicate that further investigations of low concentrations of permethrin with 10^8 and 10^9 spores/ml *M. anisopliae* are warranted in an effort to seek more effective *I. scapularis* control options. Importantly, the results indicate a lack of antagonism between low doses of permethrin and *M. anisopliae*.

Acknowledgments

We thank Dutchess County, New York, the American Lyme Disease Foundation, and the National Institutes of Health (Grant AI 40076 to RSO and

F. Keesing) for financial support. This is a contribution to the program of the Institute of Ecosystem Studies.

References

Allan S.A. and Patrican L.A. 1995. Reduction of immature *Ixodes scapularis* (Acari: Ixodidae) in woodlots by application of desiccant and insecticidal soap formulations. J. Med. Entomol. 32: 16–20.

Alves R.T., Bateman R.P., Prior C. and Leather S.R. 1998. Effects of simulated solar radiation on conidial germination of *Metarhizium anisopliae* in different formulations. Crop Protect. 17: 675–679.

Anderson T.E. and Roberts D.W. 1983. Compatibility of *Beauveria bassiana* isolates with insecticides formulations used in Colorado potato beetle (Coleoptera: Chrysomelidae) control. J. Econ. Entomol. 76: 1437–1441.

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

Beugnet F. and Chardonnet L. 1995. Tick resistance to pyrethroids in New Caledonia. Vet. Parasitol. 56: 325–338.

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

CDC (Centers for Disease Control and Prevention) 2003. Notice to readers: final 2002 reports of notifiable diseases. Morbidity and Mortality Weekly Report 8, August 5(31): 741–750.

Clark R.A., Casagrande R.A. and Wallace D.B. 1982. Influence of pesticides on *Beauveria bassiana*, a pathogen of the Colorado potato beetle. Environ. Entomol. 11: 67–70.

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

Croft B.A. 1990. Arthropod Biological Control Agents and Pesticides. John Wiley and Sons, New York, p.723.

Curran K.L., Fish D. and Piesman J. 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 Fish D. 1995. 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., Fish D. and Falco R.C. 1989. Seasonal activity and survival of adult *Ixodes dammini* (Acari: Ixodidae) in southern New York State. J. Med. Entomol. 26: 610–614.

Daoust R.A. and Roberts D.W. 1983. Studies on the prolonged storage of *Metarhizium anisopliae* conidia: effect of growth substrate on conidial survival and virulence against mosquitoes. J. Invert. Pathol. 41: 161–170.

Dumler J.S., Barbet A.F., Bekker C.P., Dasch G.A., Palmer G.H., Ray S.C., Rikihisa Y. and Rurangirwa F.R. 2001. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. Int. J. Syst. Evol. Microbiol. 51: 2145–2165.

Fernandes F.F. 2001. Toxicological effects and resistance to pyrethroids in *Boophilus microplus* from Goiás, Brazil. Arq. Bras. Med. Vet. Zootec. 53: 538–543.

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

Gassner B., Wuthrich A., Scholtysik G. and Solioz M. 1997. The pyrethroids permethrin and cyhalothrin are potent inhibitors of the mitochondrial complex I. J. Pharmacol. Exp. Ther. 281: 855–860.

Gindin G., Samish M., Zangi G., Mishoutchenko A. and Glazer I. 2002. The susceptibility of different species and stages of ticks to entomopathogenic fungi. Exp. Appl. Acarol. 28: 283–288.

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

Goodwin B.J., Ostfeld R.S. and Schauber E.M. 2001. Spatiotemporal variation in a Lyme disease host and vector: black-legged ticks on white-footed mice. Vector Borne Zoonotic Dis. 1: 129–138.

Hiromori H. and Nishigaki J. 2001. Factor analysis of synergistic effects between the entomopathogenic fungus *Metarhizium anisopliae* and synthetic insecticides. Appl. Entomol. Zool. 36: 231–236.

Johnson D.L., Smits J.E., Jaronski S.T. and Weaver D.K. 2002. Assessment of health and growth of ring-necked pheasants following consumption of infected insects or conidia of entomopathogenic fungi, *Metarhizium anisopliae* var acridum and *Beauveria bassiana*, from Madagascar and North America. J. Toxicol. Environ. Health (A) 65: 2145–2162.

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

Kaakeh W., Reid B.L., Bohnert T.J. and Bennett G.W. 1997. Toxicity of imidacloprid in the German cockroach (Dictyoptera: Blattellidae), and the synergism between imidacloprid and *Metarhizium anisopliae* (Imperfect Fungi: Hyphomycetes). J. Econ. Entomol. 90: 473–482.

Kaaya G.P. 2000. Laboratory and field evaluation of entomogenous fungi for tick control. Ann. NY Acad. Sci. 916: 559–564.

Kaaya G.P. and Hassan S. 2000. Entomogenous fungi as promising biopesticides for tick control. Exp. Appl. Acarol. 24: 913–926.

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

Lacey L.A., Frutos R., Kaya H.K. and Vail P. 2001. Insect pathogens as biological control agents: do they have a future? Biol. Control 21: 230–248.

Lecuona R.E., Edelstein J.D., Berretta M.F., La Rossa F.R. and Arcas J.A. 2001. Evaluation of *Beauveria bassiana* (Hyphomycetes) strains as potential agents for control of *Triatoma infestans* (Hemiptera: Reduviidae). J. Med. Entomol. 38: 172–179.

Lewis P., Klemm D., Lazorchak J., Norbert-King T., Heber M. and Peltier W. (eds) 1994. Short-Term Methods for Testing the Chronic Toxicity of Effluents and Surface Waters to Fresh Water Organisms, 2nd ed. EPA-600/4-91-003. Environmental Monitoring Systems Laboratory, Cincinnati, OH.

Li D.P. and Holdom D.G. 1994. Effects of pesticides on growth and sporulation of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). J. Invert. Pathol. 63: 209–211.

Miller R.J., George J.E., Guerrero F., Carpenter L. and Welch J.B. 2001. Characterization of acaricide resistance in *Rhipicephalus sanguineus* (latreille) (Acari: Ixodidae) collected from the Corozal Army Veterinary Quarantine Center, Panama. J. Med. Entomol. 38: 298–302.

Mohamed A.K., Pratt J.P. and Nelson F.R. 1987. Compatibility of *Metarhizium anisopliae* var. anisopliae with chemical pesticides. Mycopathologia 99: 99–105.

Mount G.A. 1981. *Amblyomma americanum*: area control of over-wintered nymphs and adults in Oklahoma with acaricides. J. Econ. Entomol. 74: 24–26.

Mwangi E.N., Kaaya G.P. and Essuman S. 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.

Nolan J. 1990. Acaricide resistance in single and multi-host ticks and strategies for control. Parassitologia 32: 145–153.

Onofre S.B., Miniuk C.M., de Barros N.M. and Azevedo J.L. 2001. Pathogenicity of four strains of entomopathogenic fungi against the bovine tick *Boophilus microplus*. Am. J. Vet. Res. 62: 1478–1480.

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

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

Ostfeld R.S., Schauber E.M., Canham C.D., Keesing F., Jones C.G. and Wolff J.O. 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.

Pachamuthu P. and Kamble S.T. 2000. In vivo study on combined toxicity of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) strain ESC-1 with sublethal doses of chlorpyrifos, propetamphos, and cyfluthrin against German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 93: 60–70.

Paiao J.C.V., Monteiro A.C. and Kronka S.N. 2001. Susceptibility of the cattle tick *Boophilus microplus* (Acari: Ixodidae) to isolates of the fungus *Beauveria bassiana*. World J. Microbiol. Biotech. 17: 245–251.

Pancholi P., Kolbert C.P., Mitchell P.D., Reed K.D.Jr., Dumler J.S., Bakken J.S., Telford S.R. III and Persing D.H. 1995. *Ixodes dammini* as a potential vector of human granulocytic ehrlichiosis. J. Infect. Dis. 172: 1007–1112.

Patrican L.A. and Allan S.A. 1995. Application of desiccant and insecticidal soap treatments to control *Ixodes scapularis* (Acari: Ixodidae) nymphs and adults in a hyperendemic woodland site. J. Med. Entomol. 32: 859–863.

Quintela E.D. and McCoy C.W. 1997. Pathogenicity enhancement of *Metarhizium anisopliae* and *Beauveria bassiana* to first instars of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) with sublethal doses of imidacloprid. Environ. Entomol. 26: 1173–1182.

Reis R.C.S., Melo D.R., Souza E.J. and Bittencourt V.R.E.P. 2001. *In vitro* action of the fungi *Beauveria bassiana* (Bals) Vuill and *Metarhizium anisopliae* (Metsch) Sorok on nymphs and adults of *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae). Arq. Bras. Med. Vet. Zootec. 53: 544–547.

Samish M., Gindin G., Alekseev E. and Glazer I. 2001. Pathogenicity of entomopathogenic fungi to different developmental stages of *Rhipicephalus sanguineus* (Acari: Ixodidae). J. Parasitol. 87: 1355–1359.

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

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

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

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

Schulze T.L., McDevitt W.M., Parkin W.E. and Shisler J.K. 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., Taylor G.C., Jordan R.A., Bosler E.M. and Shisler J.K. 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., Taylor G.C., Vasvary L.M., Simmons W. and Jordan R.A. 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.

Smith T.M. and Stratton G.W. 1986. Effects of synthetic pyrethroid insecticides on nontarget organisms. Residue Rev. 97: 93–120.

Soderlund D.M. and Knipple D.C. 2003. The molecular biology of knockdown resistance to pyrethroid insecticides. Insect. Biochem. Mol. Biol. 33: 563–577.

Solberg V.B., Neidhardt K., Sardelis M.R., Hoffmann F.J., Stevenson R., Boobar L.R. and Harlan H.J. 1992. Field evaluation of two formulations of cyfluthrin for control of *Ixodes dammini* and *Amblyomma americanum* (Acari: Ixodidae). J. Med. Entomol. 29: 634–638.

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

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

Stafford K.C. III 1993. Reduced abundance of *Ixodes scapularis* (Acari: Ixodidae) with exclusion of deer by electric fencing. J. Med. Entomol. 30: 986–996.

Stafford K.C. III 1997. Pesticide use by licensed applicators for the control of *Ixodes scapularis* (Acari: Ixodidae) in Connecticut. J. Med. Entomol. 34: 552–558.

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

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

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

Wilkinson L., Hill M.A. and Vang E. 1992. SYSTAT: Statistics, Version 5.2 Edition. SYSTAT, Evanston, p.724.

Zar J.H. 1999. Biostatistical Analysis, 4th ed. Prentice Hall, Englewood, NJ.

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

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