

Controlling Ticks and Tick-borne Zoonoses with Biological and Chemical Agents

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*Ticks are important vectors of disease-causing pathogens of humans, wildlife, and livestock. Reducing tick abundance is an important but elusive goal. Chemical pesticides applied to habitats occupied by ticks can be effective but appear to have significant negative effects on nontarget organisms. Devices that apply insecticides directly to vertebrate hosts for ticks reduce nontarget effects, and recent field tests support their effectiveness, but securing the devices and avoiding food subsidies to tick hosts remain significant challenges. Recent research has identified several types of organisms that show potential as biological control agents for ticks. Probably the most promising are the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*, which laboratory studies indicate are often highly lethal to several different tick species at multiple life stages. The few field tests undertaken show somewhat weaker impacts on tick survival, but suggest that the effectiveness of these fungi in controlling ticks could be enhanced by (a) identifying or selecting for highly lethal strains; (b) applying fungal spores directly to vertebrate hosts for ticks; and (c) optimizing the dose, delivery medium, and seasonal timing for environmental deployment. Thus both host-targeted chemical control and biocontrol of ticks show much promise, and would benefit from further research.*

Keywords: tick control, entomopathogenic fungi, biocontrol, biological control, Lyme disease

Ticks are obligate, blood-feeding ectoparasites of vertebrate hosts. They feed by inserting specialized piercing mouthparts (figure 1) into the skin of the host, avoiding detection by using a salivary pharmacopeia of anti-inflammatories, analgesics, antihistamines, and anticoagulants (Ribeiro et al. 1985, Sonenshine 1993). Tick life histories can be categorized by the degree of intimacy between tick and host. The nidicolous (from the Latin *nidis*, or nest) tick species, including almost all members of the family Argasidae and some members of the Ixodidae, spend their entire lives within dwellings—nests, burrows, caves—used by their hosts. The habitat specificity exhibited by nidicolous ticks typically is associated with a high degree of host specificity. The remaining, nonnidicolous tick species occupy habitats such as forests, savannas, grasslands, and shrublands, where they undergo long periods of diapause interspersed with short periods of host seeking or questing. After attaching to a host and feeding for a few days to a few weeks, these ticks drop off, spend weeks to many months digesting the blood meal, molt into the next stage, and then repeat the process with a different host. Adult ticks reproduce and die after their blood meal. A typical nonnidicolous tick is the blacklegged tick (*Ixodes scapularis*), a species widespread in eastern and central North America. The life cycle of this tick lasts more than 2 years, during which individuals take three blood meals, one each as a larva, nymph, and adult, typically from three different host species (figure 2).

Many viral, bacterial, and protozoal species have evolved to take advantage of the nonnidicolous tick life history, using the tick as a vehicle for dispersing from one vertebrate host to another. In the tick, these microbes generally reproduce little until the tick begins feeding on a host, and they typically do not cause obvious disease. In the vertebrate host, however, the microbes reproduce, disseminate, and often cause disease. Tick-borne microbial pathogens, which cause human and livestock diseases such as Lyme disease, anaplasmosis,

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Figure 1. Photomicrograph of a nymphal blacklegged tick (*Ixodes scapularis*), dorsal view. Inset shows a ventral view of the mouthparts, with the jagged-edged central hypostome and the two palps on each side. These mouthparts are embedded in the host while the tick takes a blood meal. Photomicrograph: R. Ostfeld laboratory.

ehrlichiosis, babesiosis, tick-borne encephalitis, Crimean–Congo hemorrhagic fever, Rocky Mountain spotted fever, Colorado tick fever, tick typhus, tularemia, heartwater, East Coast fever, and Nairobi sheep disease, have enormous negative impacts on human health and economic development worldwide. Because of the near absence of vaccines, inefficient diagnostic capabilities, and imperfect treatments for tick-borne illnesses, the major means of reducing the burden of tick-borne disease is reducing the abundance of ticks.

Attempts to control tick population abundance require at least two strategic decisions: what sort of control agent to use and how to deliver it. Control agents can be either chemical or biological, and delivery can be either to the environment (e.g., forest floor, pasture, lawn) where ticks seek hosts and undergo diapause or directly to the hosts on which ticks feed. In this overview, we will first briefly review the more traditional use of chemical control of ticks through environmental applications, but our treatment will be somewhat cursory, given recent reviews of this topic (Stafford and Kitron 2002). Next, we will explore more recent advances in the delivery of chemical control agents to wildlife and livestock hosts for ticks. Last, after a brief general discussion of the strengths and limitations of biological control (biocontrol), we will describe recent developments in the use of biocontrol agents deployed against ticks both off-host and on-host, ending with our view of the future prospects for tick control.

Traditional tick control with chemical insecticides

The primary means of reducing tick abundance is application of chemical insecticides into the environment where ticks seek hosts. Insecticides typically are highly lethal to ticks (although ticks are arachnids, not insects), and field applications generally are quite effective in reducing tick numbers (Sonenshine

1993, Stafford and Kitron 2002). Conventional organophosphate, carbamate, and pyrethroid insecticides generally are inexpensive, and broadcast delivery of chemical pesticides can be effective in reducing tick numbers within localized areas (Schulze et al. 1991, 2001a). Carbaryl and chlorpyrifos appear to be the most widely used insecticides for controlling ticks (Stafford and Kitron 2002), but their high toxicity to vertebrates has triggered the banning of some products by federal agencies and the discontinuation of others by manufacturers (Schulze et al. 2001a). Somewhat less toxic alternatives, including the synthetic pyrethroids, can be effective in lower doses than organophosphate and carbamate compounds, at least over the short term. For example, a single application of deltamethrin, a synthetic pyrethroid, along a band extending from a lawn–forest edge to 7 meters (m) into the forest resulted in a reduction in tick density of 90% or more over the ensuing 9 days (Schulze et al. 2001a). However, synthetic pyrethroids are not devoid of toxic effects on wild vertebrates, par-

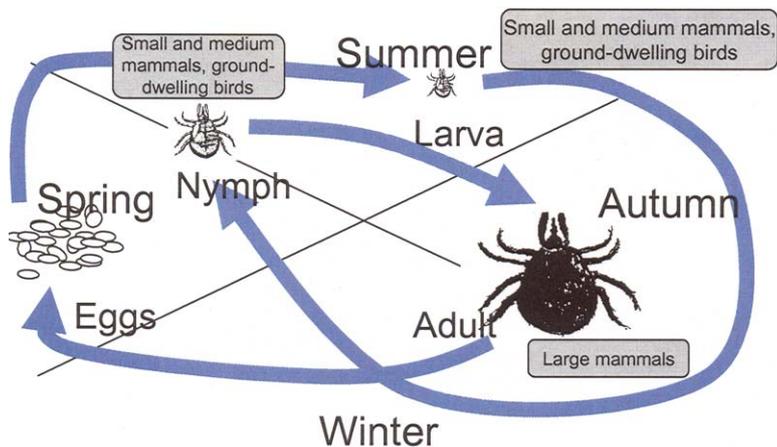


Figure 2. Generalized life cycle of the blacklegged tick in North America. The four stadia are egg, larva, nymph, and adult. The predominant hosts for each feeding stage are indicated in boxes, although larvae and nymphs in particular are known to parasitize dozens of different mammalian, avian, and reptilian hosts, and adults feed on several medium to large mammals in addition to deer. Modified from Van Buskirk and Ostfeld 1995.

ticularly when used in combination with other insecticides (Thompson 1996). In addition, all of the commercial insecticides are lethal to many invertebrates, including pollinators and predators on arthropod pests (Schauber et al. 1997). The nature and extent of undesirable, nontarget effects on invertebrates are rarely determined in field trials of the efficacy of chemical insecticides for tick control (for an exception, see Schulze et al. 2001b), but they could be considerable. Finally, repeated insecticide applications can cause the evolution of insecticide resistance (Roush 1993). These shortcomings of broadcast chemical insecticides have led to a search for alternative methods of controlling ticks.

Host-targeted tick control with chemical insecticides

Chemical pesticides delivered directly to livestock hosts (e.g., via cattle and sheep dips) have been employed successfully for many years (reviewed by George et al. 2004). Although clearly effective at reducing transmission of tick-borne pathogens to livestock, repeated heavy applications of pesticides to hosts can cause considerable mortality in nontarget arthropods through environmental contamination (Gassner et al. 1997). Moreover, evolved resistance to insecticides, which is a well-known problem with mosquitoes, is a persistent issue for tick species such as *Boophilus microplus* that are chronically exposed by virtue of their close association with cattle to which the insecticides are applied (Foil et al. 2004, George et al. 2004).

Only recently have researchers explored the delivery of pesticides directly to wildlife hosts for ticks as a means of reducing the risk of tick-borne human illness. Targeting wildlife hosts of ticks with insecticides poses many challenges, owing to the variety of vertebrate hosts for many species, their ability to disperse, the potential impact of insecticides or bait attractants on host populations, and the potential proximity of hosts to residential areas. For blacklegged ticks in North America, new devices that deliver insecticide to hosts for adult ticks (largely deer), or to hosts for immature ticks (largely rodents), are currently being tested (table 1).

The four-poster deer feeder is a device that attracts deer to a bin of corn; to feed on the corn, the deer must rub their heads and necks against paint rollers strategically placed around the bin and impregnated with insecticide. Two recent field studies designed to assess the impacts of four-posters on the abundance of blacklegged ticks in Lyme disease–endemic areas of Maryland concluded that tick populations were reduced substantially following several years of continuous deployment (Carroll et al. 2002, Solberg et al. 2003). Carroll and colleagues (2002) found that three sites at which 25 four-posters per site had been operated for 5 years had densities of nymphal blacklegged ticks 69%, 76%, and 80% lower than on unmanipulated control sites. The study by Solberg and colleagues (2003), which consisted of one treatment and one control (untreated) site, also claimed a reduction in tick numbers, but that work was unreplicated. Moreover, tick numbers were markedly low on the treatment site before the deployment of four-posters, weakening the conclusions drawn. To date, no studies have been performed in the suburban land-

scapes where most cases of Lyme disease occur (Barbour and Fish 1993). The difficulty of keeping nontarget animals such as raccoons and children from visiting the four-posters, and the high cost of installing and maintaining these devices, are important challenges for this technique. Another obstacle is that the detection of chronic wasting disease (a degenerative brain disease caused by aberrant proteins called prions) in deer within Lyme disease–endemic zones has led some states to ban the feeding of deer. Despite the potentially high efficacy of four-posters, the feeding ban, intended to reduce deer-to-deer transmission of prions, makes widespread use of these devices unlikely.

Two devices for delivering insecticide to small rodents are commercially available. One consists of a cardboard tube with permethrin-impregnated cotton that can be retrieved by rodents to their nests. Field tests of this product have produced mixed results, but generally do not support its efficacy in reducing numbers of host-seeking ticks (Stafford and Kitron 2002). An early study conducted in coastal Massachusetts (Mather et al. 1987) documented a marked reduction in the numbers of immature blacklegged ticks on white-footed mice in areas provided with abundant bait tubes, compared with untreated areas, but the impact of the bait tubes on host-seeking nymphal ticks was not assessed. This is unfortunate, because host-seeking nymphal ticks are primarily responsible for transmission of Lyme disease spirochetes and other pathogens to humans. Another test of this product in coastal Massachusetts (Deblinger and Rimmer 1991) consisted of the deployment of 2000 tubes in a 7.3-hectare site over 3 years. Although the apparent lack of host-seeking nymphs and of complaints about human tick bites was interpreted as confirmation of the efficacy of the product, the lack of replication and of control sites weakens this claim. The most comprehensive field deployments of these tubes over multi-year periods in Connecticut and New York showed no significant reduction in numbers of host-seeking ticks (Daniels et al. 1991, Stafford 1992).

The other device is a small plastic box (“bait box”) with holes that allow access by small rodents. Rodents are attracted by a food source within the box, but to reach the food they must contact a wick that applies fipronil, a pesticide marketed as “Frontline.” A field test of this product, conducted by the device’s inventors on more than 300 different properties in Connecticut, supported its efficacy. Numbers of host-seeking nymphal ticks, a key risk factor for tick-borne diseases, were reduced by more than 50% on bait box–treated properties compared with untreated properties (Dolan et al. 2004). Unfortunately, as in one of the four-poster studies described above, considerably lower numbers of ticks on the treatment sites preexisted the deployment of bait boxes (Dolan et al. 2004), suggesting that conditions unrelated to the bait box treatment may have played a role in the apparent reduction in tick numbers caused by the device. A clearer result of deploying bait boxes, however, was the reduction in the proportion of nymphal blacklegged ticks infected with *Borrelia burgdorferi* (the spirochete that causes Lyme disease) and

Anaplasma phagocytophilum (the bacterium that causes human granulocytic ehrlichiosis, or anaplasmosis) by 67% and 64%, respectively. Reduced infection prevalence in nymphal ticks would serve to reduce risk of human exposure to tick-borne infections, and would be expected from a device that preferentially targets ticks on rodents, which are the principal reservoirs for these pathogens.

Although host-targeted chemical insecticides show promise as a means of reducing the abundance of ticks, more thorough testing of safety and efficacy will be required. The efficacy of deer- or rodent-targeted interventions will undoubtedly be enhanced by high, even saturation, densities of devices. However, the health consequences of massively supplementing food for either rodents or deer are unknown. Certainly, supplemental foods can induce strong population growth in rodents (Boutin 1990), with unpredictable net effects on disease transmission. The potential impact of seasonally or chronically available supplemental corn on deer population dynamics is unknown. In addition, very little is known about the degree to which host-targeted pesticides and their toxic breakdown products accumulate in avian and mammalian predators and scavengers on the target organisms.

Classical and augmentative biocontrol

The most promising alternatives to chemical pesticides are biological control (biocontrol) agents, which are species that consume target pest organisms via predation, herbivory, or parasitism. Biocontrol agents typically are nontoxic to humans and to nontarget wildlife (for a few exceptions, see below). Moreover, biocontrol agents are expected to coevolve with their target organisms, reducing the likelihood that resistance will evolve. Although biocontrol programs have a mixed record of success and include some spectacular failures (e.g., the decimation of island endemic birds by mongooses [*Herpestes javanicus*] released to control introduced rats), biocontrol appears promising, but understudied, for the control of ticks.

The predominant form of biocontrol is “classical biocontrol,” whereby nonnative predators, herbivores, or parasites (including parasitoids and pathogens) are introduced to control nonnative pest species. The vast majority of classical biocontrol efforts have been directed at exotic plants and insect pests of agricultural products. The most widely recognized danger of classical biocontrol is that the biocontrol agent will attack nontarget organisms, particularly native taxonomic relatives of the exotic target species (Stiling 2004, Louda et al. 2005). Attacks on nontarget species are expected to be minimal when the biocontrol agent is a specialist on the target species, and much effort is devoted to confirming that a potential biocontrol agent is indeed a specialist on the target pest. Unfortunately, many biocontrol agents identified during prerelease screening as specialists have attacked nontarget organisms once they were released into new ecological communities with alternative hosts, particularly when the target pest became scarce (Lynch et al. 2002). In addition, owing to their dependence on the target organism, specialist biocontrol agents are likely to become ineffective or disappear entirely

when the abundance of target organisms declines, and this interdependence can cause damaging fluctuations in the abundance of the pest. Other dangers include displacement of native predators or parasites on the pest species, for example, when native ladybird beetles are displaced following introduction of exotic ladybirds to control aphids (Evans 2004).

Some biocontrol agents unintentionally provide large food subsidies to predators, with potentially serious consequences. For instance, native deer mice (*Peromyscus maniculatus*) feast on gall flies (*Urophora*), which were released to control noxious knapweeds (*Centaureia*). Deer mice with access to this unintended food supplement can undergo explosive population growth (Ortega et al. 2004), with possible negative consequences for human health, given that high deer mouse density is correlated with high hantavirus disease incidence in humans (Yates et al. 2002).

Despite these shortcomings, classical biocontrol has seen some well-known successes, such as the use of myxoma virus to control rabbits introduced to Australia (Hayes and Richardson 2001) and the resurgence of populations of *Entomophaga maimaiga*, a fungal pathogen of gypsy moths (*Lymantria dispar*), nearly 100 years after its release (Hajek et al. 1990). Efforts to reduce the probability of unpleasant surprises are ongoing (Louda et al. 2003, Kimberling 2004).

Far less attention has been paid to “augmentative biocontrol,” whereby native predators, herbivores, or parasites are used to control a target pest species. Augmentative biocontrol is typically attempted with insect predators or parasitoids, reared in insectaries, that are released in massive numbers (“inundative” biocontrol) to suppress insect pests (Collier and Van Steenwyk 2004). A major benefit of augmentative biocontrol is that it does not involve introducing species outside their native geographic ranges, and thereby avoids problems that accompany species introductions. Its major limitation arises from the inability of the native natural enemy to control the pest without being augmented repeatedly. In other words, if the pest and control agent coexist naturally, but the pest (by definition) is normally not controlled by the agent, the effectiveness of the control agent seems in doubt. Poor control under normal (unaugmented) conditions, however, can be caused by low natural contact rates between the control agent and the target. A key challenge for augmentative biocontrol is to alter the abundance or distribution of the control agent to maximize contact rates with the target species.

Use of biological agents to control ticks

Natural enemies of ticks include insectivorous birds, parasitoid wasps, nematodes, *Bacillus thuringiensis* bacteria, and deuteromycete fungi (largely *Metarhizium anisopliae* and *Beauveria bassiana*) (Samish and Rehacek 1999). The potential of each of these taxa as biocontrol agents will be discussed in turn.

Mammals and birds typically consume ticks during self-grooming. For example, laboratory studies demonstrate that significant numbers of larval blacklegged ticks are consumed by white-footed mice (*Peromyscus leucopus*) during self-

grooming (Shaw et al. 2003). Nevertheless, a high proportion of ticks encountering mice survive and feed to repletion, and abundance of blacklegged ticks is positively correlated with that of mice (Ostfeld et al. 2001). Some vertebrates attack ticks in the environment. Wild turkeys (*Meleagris gallopavo*) consume a very high proportion of the immature blacklegged ticks they encounter while grooming (Ostfeld and Lewis 1999), and might reduce tick numbers. In the case of host species, such as turkeys, that groom a high proportion of the ticks that attempt to feed from them, abundance of ticks could be suppressed by high host abundance. To our knowledge, such negative correlations between the abundances of specific hosts and ticks have not been explored. Unfortunately, enhancing the numbers or distribution of turkeys as biocontrol agents seems infeasible. Moreover, turkeys are an important host for lone star ticks (*Amblyomma americanum*) (Kollars et al. 2000), and high abundance of turkeys could facilitate populations of this tick species, which is a vector of human monocytic ehrlichiosis.

Other birds also consume host-seeking ticks in the environment. On the basis of a modest study using small enclosures and exclosures to manipulate helmeted guineafowl (*Numida meleagris*) on lawns (Duffy et al. 1992), this bird has reached cult status as a biocontrol agent for blacklegged ticks and is credited with reducing the transmission of Lyme disease bacteria to people (figure 3). However, tick reduction by guineafowl was restricted to the adult stage (Duffy et al. 1992), which transmits a small minority of Lyme disease cases to people (Barbour and Fish 1993), and to lawns, which maintain far smaller populations of ticks than do brushy and wooded habitats (Ostfeld et al. 1996). Recently, we extended Duffy and colleagues' (1992) study by comparing numbers of adult and nymphal blacklegged ticks on properties with and without free-ranging guineafowl in a highly Lyme disease–endemic zone in southeastern New York State. We found that although reduced abundance of adult ticks in the presence of guineafowl suggested that the birds do attack this life stage, the presence of guineafowl did not significantly reduce the density of nymphs (box 1), which transmit the vast majority of Lyme disease cases (Barbour and Fish 1993). We hypothesize that the provision of food (grain) to guineafowl attracts small rodents, which might import immature ticks onto properties containing the birds, and that this might counteract the suppressive effects of predation by the fowl on adult ticks.

The most obvious vertebrate consumers of ticks are oxpeckers (*Buphagus* spp.), pan-African birds that specialize on ticks feeding on both wild and domestic large mammals. The daily intake of ticks by oxpeckers is reported to be in the hundreds (adult ticks) to thousands (nymphs) (Samish 2000). However, neither a reduction in tick populations by natural populations of oxpeckers nor the feasibility of augmenting their numbers has been demonstrated.

Parasitoid wasps and flies are among the most effective agents in the biocontrol of insects (Kimberling 2004). Parasitoids are often, although not always, specialists on one host

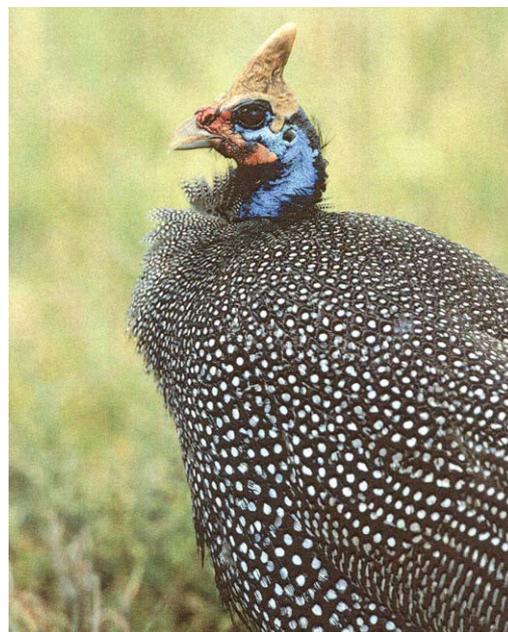


Figure 3. The helmeted guineafowl (*Numida meleagris*). The cult status of this bird as a tick predator, and hence a protector of people from exposure to tick-borne infections, appears to be unwarranted. Although evidence suggests that these birds eat adult ticks, they appear not to reduce the numbers of nymphs (the stage responsible for most cases of Lyme disease) sufficiently to have a strong protective effect. Photograph: WildeyesImages.com.

taxon (or a small number of host taxa), and typically have sufficiently high fecundity to allow rapid population response to host abundance. Both of these features enhance their potential as control agents. The chalcid wasp, *Ixodiphagus hookeri*, a parasitoid that specializes on ixodid ticks, was introduced to Massachusetts islands and the northwestern United States several times in the early 20th century to control populations of the American dog tick, *Dermacentor variabilis*, and the Rocky Mountain wood tick, *Dermacentor andersoni*. Although systematic monitoring following these mass releases was not conducted, spotty sampling revealed that the parasitoid became established in the blacklegged tick population on Naushon Island, Massachusetts (Mather et al. 1987). Neither immediate nor long-term control of ticks was reported, however, and recent studies suggest that *I. hookeri* will persist only when blacklegged ticks and their deer hosts are hyperabundant, which tends to occur on islands and adjacent mainland sites in southern New England (Stafford et al. 2003). The reported threshold effect of host abundance suggests that, in fact, tick populations control those of parasitoids from the bottom up, rather than parasitoids exerting top-down control of their hosts. Nevertheless, inundative releases of *I. hookeri* have shown promise in controlling tick populations infesting cattle in Kenya (Mwangi et al. 1997), and a modeling study by Knipling and Steelman (2000) suggests that massive environmental releases of

laboratory-reared parasitoids could reduce tick populations locally. Unfortunately, the production of inundative quantities of *I. hookeri* might be prohibitively expensive, given the high costs of maintaining tick colonies, which would be necessary to raise these obligate tick parasitoids.

Several species of steinernematid and heterorhabditid nematodes, including those used commercially to control insect pests, are also pathogenic to ticks. Juvenile nematodes invade ticks either through natural orifices or using digestive enzymes and mechanical force to penetrate the host cuticle. Once in the host haemocoel, they release mutualistic bacteria that attack and kill the tick (Zhioua et al. 1995). The pathogenicity of nematodes is generally restricted to engorged adult female ticks (Samish and Glazer 2001), probably due to ease of access through the genital pore or through stretched and thin cuticle. However, because these nematodes do not complete their life cycles within the tick host (Samish and Glazer 2001), ticks are incapable of sustaining nematode populations in either laboratory or natural environments. Moreover, commercially available entomopathogenic nematodes appear not to survive winter temperatures within temperate regions where many tick-borne diseases of humans occur (Samish and Glazer 2001). As is the case for parasitoids, release of nematodes to control ticks is likely to be successful only temporarily and in highly localized areas. However, systematic searches may identify native entomopathogenic nematodes with greater potential as biocontrol agents. Potentially, native entomopathogenic nematodes could use nontick arthropods as reservoir hosts (Donald Strong, Bodega Marine Laboratory, University of California, Davis, personal communication, 6 August 2005) and opportunistically attack ticks during the postfeeding diapause.

Although several bacterial species are pathogenic to ticks, the usefulness of bacteria as biocontrol agents is poorly studied. *Bacillus thuringiensis*, which is used as a biocontrol agent for many insects, is pathogenic to ticks, but apparently must be ingested to be effective (Samish and Rehacek 1999, Zhioua et al. 1999). Because ticks tend to ingest only host blood, inducing ticks to ingest these bacteria seems impractical, and

the prospects for *B. thuringiensis* as a biocontrol agent seem poor. Recent surveys of microbes naturally infecting black-legged ticks and American dog ticks (Martin and Schmidtman 1998, Grindle et al. 2003) reveal a rich flora including spore-forming and crystal-forming bacteria that, if found to be entomopathogenic, could be developed as potential biocontrol agents.

Biocontrol of ticks using entomopathogenic fungi

Dozens of species of eumycete and deuteromycete fungi are known to attack and kill ixodid ticks, but only a handful of species have been extensively studied. *Metarhizium anisopliae* (figure 4) and *Be. bassiana*, in particular, appear to cause significant tick mortality in laboratory assays. Both of these taxa have virtually worldwide distributions (Humber 1992), although much genetic variation exists among geographic areas. Fungus-caused mortality varies substantially among different tick species and life stages, fungal taxa, and modes of application. Mortality tends to be higher in engorged or engorging ticks than in unfed ticks; higher in adults than in nymphs or larvae; higher in *Ixodes* than in *Boophilus*, with *Amblyomma* and *Rhipicephalus* highly variable; higher in oil-based than in water-based delivery mode; and higher in laboratory than in field assays. (A table reviewing responses by 12 species of ticks to 9 species of entomopathogenic fungi is available from the authors.) The mechanisms that underlie these sources of variation are poorly understood.

Numerous laboratory assays of both *M. anisopliae* and *Be. bassiana* support their lethal effects on several tick species of epidemiological and veterinary importance. In addition, strong and demographically significant sublethal effects of these fungi on ixodid ticks have been noted by several researchers (reviewed in Hornbostel et al. 2004). A combined field and laboratory study using *M. anisopliae* on *Ixodes scapularis* revealed that fungal exposure reduced the body mass of engorging female ticks and the mass of their egg clutches by up to 50% (Hornbostel et al. 2004). Engorged larvae and nymphs treated with fungus molted into significantly lighter nymphs and adults, respectively, compared to untreated con-

Box 1. A field study of the effects of guineafowl on blacklegged ticks.

In the eastern and central United States, the blacklegged tick, *Ixodes scapularis*, is the primary vector of *Borrelia burgdorferi*, *Babesia microti*, and *Anaplasma phagocytophilum*, the etiological agents of Lyme disease, babesiosis, and human granulocytic ehrlichiosis, respectively. The burden of all three diseases could potentially be alleviated by reducing the abundance of the tick vector, particularly in peridomestic settings where exposure can be high (Barbour and Fish 1993). The use of insectivorous helmeted guineafowl (*Numida meleagris*) for tick control is an increasingly popular tactic, widely promoted in newsletters and Web sites dedicated to Lyme disease. The promotion of guineafowl appears to be based largely on the study by Duffy and colleagues (1992), who compared the abundances of adult blacklegged ticks inside and outside small enclosures (containing guineafowl) and enclosures placed on lawns, and concluded that the presence of guineafowl was associated with lower tick density. As recognized by the authors, the study by Duffy and colleagues was limited in its ability to assess the impacts of guineafowl on disease transmission, because the research (a) was restricted to lawns, whereas tick abundance is dramatically higher in forested, ornamental, and ecotonal habitats (Ostfeld et al. 1996), and (b) assessed only the abundance of adult ticks, which transmit a small minority of Lyme disease cases (Barbour and Fish 1993).

To extend the scientific assessment of the effectiveness of helmeted guineafowl in reducing the risk of human exposure to tick-borne infections, we selected matched domestic properties in Dutchess County, New York, with and without free-ranging guineafowl, and sampled the abundance of both nymphal and adult ticks in major habitat types on these properties. Ten "treatment" properties were
(continued)

Box 1. (continued)

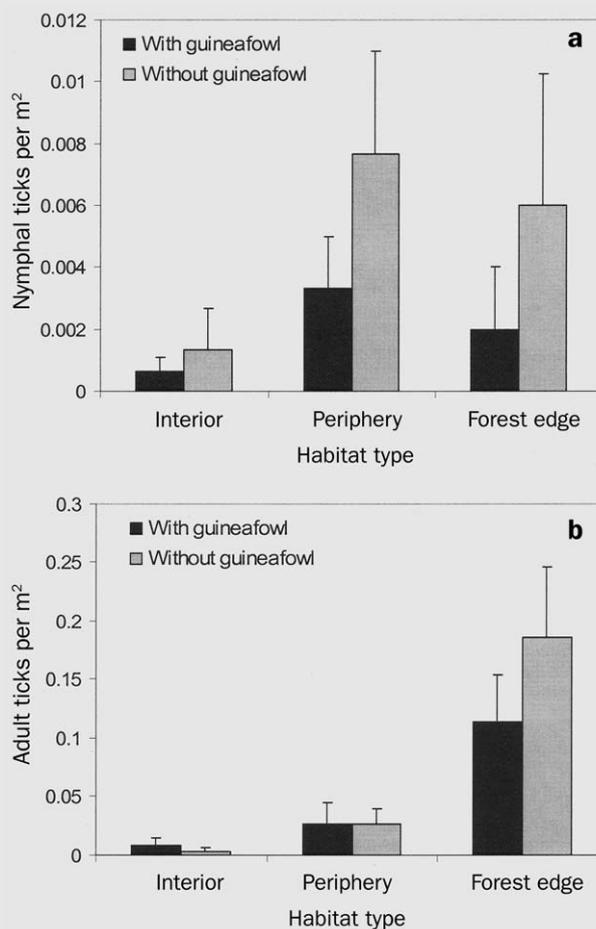
chosen on the basis of the following criteria: free-ranging guineafowl had been present for over a year, no other tick control methods were used, and no other domestic fowl were present. Each treatment property was paired with a control property located within 1 kilometer but not directly adjacent to the treatment property, to ensure that there was no spillover guinea fowl activity. Control properties fulfilled the same criteria as treatment properties, except that no guineafowl were present. Treatment and control sites were in similar landscapes and had similar compositions, with lawns, ornamental vegetation, and forested habitat. We sampled tick abundance by dragging a 1-square-meter (m^2) white corduroy cloth along premeasured transects in each of three habitat types: the lawn interior, the lawn periphery 1 to 2 m from the forest edge, and the forest periphery 3 to 5 m from the lawn edge. Sampling at each site occurred once a week for 3 weeks during the seasonal peak in nymphal host-seeking activity (20 June–10 July 2004) and again during the peak in adult activity (16–31 October 2004).

Nymphal tick densities were extremely low in lawn interiors, and somewhat higher at the lawn periphery and within the forest edge (see the figure). Despite an apparent trend toward lower nymphal densities in all three habitat types in properties with guineafowl, these differences were not statistically significant. We observed no significant difference between total tick densities on properties with and without guineafowl (Wilcoxon z value = 1.26, $p = 0.21$).

Densities of adult ticks were extremely low in lawn interiors and lawn peripheries, and considerably higher within the adjacent forest (see the figure). The density of adult ticks in properties with guineafowl was significantly lower than adult tick density in control properties (Wilcoxon z value = 2.03, $p = 0.043$). This difference was due entirely to lower abundance in the forest edge habitat.

These results, combined with the experimental study by Duffy and colleagues (1992), suggest that guineafowl actively consume adult, but not nymphal, blacklegged ticks. Nymphs are probably too tiny to be sought or detected by these birds. Chronically reduced density of adult ticks could cause reduced abundance of nymphs even without direct attacks on nymphs by guineafowl. This could occur via a reduction in the number of adult females ovipositing on properties with guineafowl. However, reduced abundance of adult ticks was not associated with significant reduction in abundance of nymphs, the stage responsible for transmitting the vast majority of Lyme disease cases (Barbour and Fish 1993). Guineafowl foraging behavior in peridomestic settings appears to be highly localized and largely confined to lawns and adjacent forests. Adult female ticks that survive predation pressure by guineafowl, find a host, and feed to repletion are likely to be transported to sites for oviposition away from the location at which they quested. Consequently, we expect local density of adult ticks to have little impact on the future density of immature stages, including nymphs.

It is possible that, in fact, guineafowl do consume nymphal ticks, but our failure to detect a significant effect on nymphal abundance was due to a concurrent increase in immigration of immature ticks on mobile hosts. According to this scenario, the provision of food (grain) for guineafowl might attract granivorous rodents, such as white-footed mice and eastern chipmunks, from adjacent forests into forest-lawn edges or the lawns themselves. If this attraction occurred during the season of larval feeding, replete larvae could drop off hosts and molt into nymphs, which would later quest on these edges or lawns. Such an importation conceivably could counteract local reduction in numbers due to predation by guineafowl. Alternatively, the lack of statistical significance might be a consequence of a relatively small sample of properties. A power analysis indicated that, if the magnitude of the difference in nymphal density between properties with and without guineafowl were maintained, a sample of 27 pairs of properties would result in significance at the 0.05 level. In sum, our results weaken the argument that guineafowl are an effective means of biologically controlling human risk of exposure to tick-borne pathogens. Modest and statistically insignificant reductions in nymphal tick abundance, combined with the potential for food provision to attract small mammalian hosts, indicate that popular recommendations to use these birds to protect human health are not supported.



Mean (+ 1 SE [standard error]) density of (a) nymphal and (b) adult blacklegged ticks within three different habitat types on properties that either did or did not maintain free-ranging guinea fowl. The three habitat types correspond to the interior of lawns, the lawn periphery within 1 to 3 meters (m) of the forest edge, and the forest edge within 3 to 5 m of the lawn. The reduction in density of host-seeking adult ticks was statistically significant, but that of host-seeking nymphs was not.

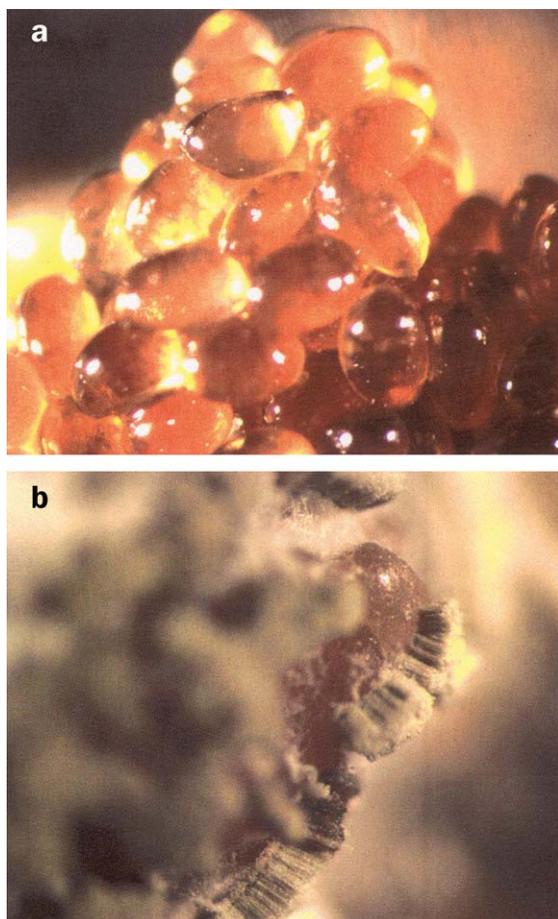


Figure 4. Egg masses produced by blacklegged ticks in the laboratory. (a) Eggs produced by a female that had not been treated with the fungus *Metarhizium anisopliae*; these eggs hatched normally. (b) Eggs produced by a female that had been treated with *M. anisopliae*; these eggs died. Photographs: R. Ostfeld laboratory.

trols. These results suggest that these fungal pathogens reduce tick fitness (fecundity and body mass), with likely negative impacts on population growth beyond that imposed by direct mortality alone.

Field deployments of both fungal species in mesh bags in pastures and directly on cattle in Kenya and Brazil suggest that these biocontrol agents have great promise in reducing tick burdens on livestock (Kaaya et al. 1996, Bittencourt 2000, Kaaya and Hassan 2000). Mortality rates tend to be moderate to high for adult and immature livestock ticks from the gen-

era *Boophilus*, *Rhipicephalus*, and *Amblyomma* exposed to entomopathogenic fungi in pastures or stables. The potential for replacing livestock dips using chemical insecticides with those employing fungal spores in solution seems quite high. We are not aware of any demonstrations of toxic effects of these fungal solutions on livestock or other terrestrial vertebrates, although nontarget effects of *M. anisopliae* and *Be. bassiana* on fish eggs have been reported (Genthner and Middaugh 1995). Isolated cases of human disease caused by entomopathogenic fungi have been reported, especially in immunocompromised individuals (DeGarcia et al. 1997, Tucker et al. 2004).

Broadcast delivery of *Be. bassiana* and *M. anisopliae* to pastures can dramatically reduce the population density of ticks known to infest cattle in Brazil (Bittencourt 2000) and Kenya (Kaaya 2000, Maranga et al. 2005). For example, Kaaya (2000) found a five- to tenfold reduction in the numbers of ticks on cattle following the spraying of fungi on pasture vegetation, and Maranga and colleagues (2005) demonstrated a synergistic effect of adding a “cocktail” of both species, compared with either species alone. To our knowledge, only two studies have employed broadcast delivery of fungal spore solution into forested habitat to assess its potential in controlling ticks. Benjamin and colleagues (2002) sprayed aqueous solution of *M. anisopliae* spores onto forest understory vegetation at a site in southeastern New York State where Lyme disease is hyperendemic. They sprayed in autumn, to coincide with the peak activity period for adult blacklegged ticks, and targeted the understory rather than the forest floor because adult blacklegged ticks tend to climb up vegetation to a height of a meter or more to seek a host. Autumn spraying and avoidance of the forest floor were also thought to minimize impacts on nontarget arthropods. After field spraying, exposed ticks retrieved to the laboratory experienced a 53% mortality rate, as compared with control ticks (sprayed with water only), which experienced only 3% mortality (Benjamin et al. 2002). A follow-up study (Hornbostel et al. 2004) demonstrated that similar field spraying of *M. anisopliae* solution resulted in 36% control of adult ticks, where the percentage of control is defined as $[1 - (n \text{ in treatment group after treatment} / n \text{ in control group after treatment})] \cdot 100$ (Abbott 1925). This degree of control via direct mortality, combined with potent sublethal effects on ticks, suggests that field spraying of fungus solution has promise as a means of substantially reducing tick abundance. The use of oil-based fungal solutions for field applications, and additional spraying during spring and summer to target immature ticks, seems likely to improve

Table 1. Recently developed devices for applying insecticides to hosts for blacklegged ticks (*Ixodes scapularis*), which are vectors of the agents of Lyme disease, anaplasmosis, ehrlichiosis, and babesiosis.

Device	Targeted tick stage	Targeted host	Attractant	Control agent	Limitations
Four-poster	Adult	Deer	Food	Permethrin or amitraz	Safety for wildlife and people, efficacy, cost
Cardboard tube	Larvae and nymphs	Rodents	Nest materials	Permethrin	Efficacy
Bait box	Larvae and nymphs	Rodents and shrews	Food	Fipronil	Efficacy, cost

efficacy, although this regimen could increase nontarget effects. In addition, the use of entomopathogenic fungi supplemented with low doses of pyrethroids or other chemical insecticides might enhance tick control without the need to apply large quantities of chemical agents. Hornbostel and colleagues (2005) demonstrated that, although an expected synergistic effect of *M. anisopliae* and permethrin on blacklegged tick mortality did not occur, these two agents did not interfere with each other's impact on ticks.

The potential drawbacks of broadcast applications of fungal biocontrol agents need to be assessed more fully. Laboratory studies indicate that entomopathogenic fungi are likely to have nontarget effects (Ginsberg et al. 2002), but the scope and magnitude of these effects in the field are not well understood. In addition, the strains of fungi released at a site are rarely, if ever, demonstrated to occur naturally at that site. The impacts of releasing exotic genotypes of a native species are unknown. For certain fungal strains, spore viability may degrade rapidly in direct sunlight, potentially reducing the effectiveness of broadcast applications in summer, when immature ticks are most abundant. Optimal temperature and moisture conditions also may be required for infection. Finally, although the evolution of resistance by ticks to entomopathogenic fungi might appear unlikely, we are not aware of any direct tests.

With the exceptions of the Kenya (Kaaya et al. 1996, Kaaya and Hassan 2000) and Brazil (Correia et al. 1998) studies on livestock mentioned above, application of entomopathogenic fungi directly to hosts has not been undertaken systematically. As for the host-targeted chemical agents described above, a major challenge for host-targeted biocontrol agents is efficient delivery to hosts while avoiding food subsidies that might boost host numbers. To assess the ability of entomopathogenic fungi applied to wildlife hosts to control tick populations, Hornbostel and colleagues (2005) used wood nest boxes attached to tree trunks at a height of 1.5 m as a platform for delivering fungal solution to white-footed mice. These nest boxes (figure 5; described in Burns et al. 2005) are known to be used by mice as supplements to natural nesting sites in underground burrows or tree holes. Hornbostel and colleagues (2005) periodically sprayed aqueous fungal solution onto cotton nesting material within the nest boxes and monitored the effects, both on the tick burdens on rodent hosts and on the abundance of host-seeking ticks, after a 1-year lag. The authors expected that the dark nest boxes would promote spore longevity and that prolonged contact of moist nesting material with tick hosts would encourage fungal infection. Although laboratory treatment of cotton nesting materials with identical fungal solution was highly lethal to larval blacklegged ticks engorging on white-footed mice, field applications were only modestly effective. Abundance of nymphal ticks in 2003, following nest-box spraying targeting larval ticks in 2002, was significantly reduced only in localized areas around the nest boxes most heavily used by mice (Hornbostel et al. 2005). We expect that the effectiveness of nest-box targeted biocontrol could be strongly enhanced by creating a saturated abundance



Figure 5. A nest box (top) attached at chest height to a tree on an experimental plot at the Institute of Ecosystem Studies in Millbrook, New York. These nest boxes are frequently colonized by white-footed mice (*Peromyscus leucopus*, below), which are a primary host for larval blacklegged ticks and the principal natural reservoir for Lyme disease spirochetes (*Borrelia burgdorferi*). Treating the cotton nesting material in these nest boxes with *Metarhizium anisopliae* resulted in modest, local reductions in the abundance of nymphal ticks. Photographs: R. Ostfeld laboratory.

of nest boxes (only about 35% of mice in field plots were known to use the nest boxes), and by deploying this method in suburban forests where mice tend to be abundant and relatively few alternative hosts for larval ticks occur.

Prospects for the future

Each of the tick-control methods described herein has strengths and limitations. Numerous studies have demonstrated the efficacy of broadcast and host-targeted chemical pesticides for tick control. The potential drawbacks of this approach include damage to nontarget organisms, the development of insecticide resistance, and human health consequences. The nontarget mortality and toxicity of chem-

ical insecticides can be reduced by avoiding organophosphate and carbamate compounds and by applying insecticides directly to hosts. Also, for multihost ticks, rapid development of insecticide resistance seems less likely than in shorter-lived arthropods or in single-host ticks facing constant chemical exposure. We suspect that, in the case of *Ixodes* vectors of human pathogens, a combined strategy of applying insecticides to deer (e.g., via four-posters) and rodents (e.g., via bait boxes) might be highly effective with limited collateral damage.

More generally, the use of integrated pest management against ticks should be pursued more vigorously. For tick vectors of livestock pathogens that plague tropical and subtropical areas, an important issue is whether the application of entomopathogenic fungi instead of chemical agents will reduce or eliminate the evolution of resistance by ticks. If so, the means of making fungal agents affordable and widely available should be pursued. Prospecting for additional biocontrol agents would seem a promising direction for further research. The primary reasons for testing *B. thuringiensis*, *M. anisopliae*, and *Be. bassiana* against ticks appear to be their known efficacy against other arthropods and their commercial availability, rather than their primacy as tick control agents or evidence of their ability to control ticks in nature. Rarely are acarologists or medical entomologists aware of the major causes of mortality in tick populations, and pursuit of these causes might reveal heretofore unrecognized natural enemies. In particular, fungal or bacterial taxa that naturally infect ticks and that might be mildly pathogenic at natural abundances might have promise as biocontrol agents, particularly if they can be deployed inundatively.

Although considerable genetic variation is known to occur within species used as biocontrol agents (Freimoser et al. 2003), little use has been made of this information for the control of ticks. For example, genetic strains of *M. anisopliae* that differ in host specificity are characterized by strong variation in the complex set of proteins they secrete to invade and kill arthropod hosts (Freimoser et al. 2003). Strains of *M. anisopliae* show particularly dramatic genetic and phenotypic variation in the expression of subtilisin proteinases, which are used to penetrate arthropod cuticles and release nutrients (Freimoser et al. 2005). The potential for either discovering natural strains or artificially selecting strains with extremely high pathogenicity to ticks (but low pathogenicity to insects) seems high. The effectiveness of strain combinations, as opposed to single strains, has not to our knowledge been tested in the lab or field.

Application of biocontrol agents to white-tailed deer as a means of reducing Lyme disease risk has not been attempted but seems to hold promise, particularly in light of successes with biocontrol agents delivered to livestock. In general, adult ticks in the process of engorging on host blood are most susceptible to attack by nematodes, bacteria, and fungi, and deer-targeted delivery would take advantage of this vulnerability. Delivery systems to hosts for adult ticks that avoid nutritional subsidies (for example, those deployed along deer

trails or in areas used for resting) are likely to be more palatable to wildlife management agencies than are those that accompany feeding stations. Similarly, biocontrol delivery systems to hosts for immature ticks that avoid nutritional subsidies (for example, those using collectible nesting materials or nest boxes) should be pursued.

Both *M. anisopliae* and *Be. bassiana* appear largely nontoxic to people and to other terrestrial vertebrates, but their impacts on nontarget arthropods and aquatic vertebrates have not been addressed adequately in the field. Further studies of the efficacy of broadcast spraying of fungal spore solution in reducing tick numbers, combined with monitoring of impacts on other arachnids, insects, and other nontarget taxa, should be undertaken. Continued research is needed into methods of maximizing contact rates between these fungi and ticks while minimizing contact rates between fungi and nontarget organisms.

In our opinion, the efficacy of *M. anisopliae* and *Be. bassiana* as tick control agents has been sufficiently well established to warrant aggressive pursuit of efficient and safe delivery systems. Prospecting for or selecting fungal strains with high specificity toward particular target tick species will maximize control while minimizing nontarget effects. Targeted research and development of deployment strategies, whether areawide, host targeted, or a combination of both, should be undertaken. Timing the deployment seasonally to coincide with vulnerable life stages of ticks, with periods when risk to nontarget organisms is minimized, or with conditions favoring maximum longevity of fungal pathogens should be a part of any strategy. The promise of tick biocontrol using entomopathogenic fungi, which has also been supported by recent studies on *Anopheles* mosquitoes (Blanford et al. 2005, Scholte et al. 2005), can only be realized with renewed commitment and strong financial support.

Conclusions

Reducing tick abundance is likely to remain the most effective method for preventing tick-borne diseases. Most recent research on reducing tick numbers has focused on the targeted delivery of chemical insecticides to particular hosts. These methods seem promising, but more rigorous tests are needed. Several methods of biocontrol of ticks, including parasitoids and some bird predators, have been shown to reduce tick numbers in some situations. Perhaps the most promising method of biocontrol is the targeted use of fungal pathogens, which has been shown to reduce tick numbers both directly (through mortality) and indirectly (through reductions in fitness). These preliminary successes demonstrate the importance and potential of rigorous research into novel and existing methods of biological control of ticks.

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