

**Tick Burdens of *Peromyscus leucopus*
and *Borrelia* Infection Rates in Ixodid Ticks
in a Fragmented Virginia Landscape**

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ABSTRACT

The emergence of southern tick-associated rash illness (STARI) has thrown into question the validity of reported Lyme disease cases throughout the southern United States. The symptoms of both are identical and a rapid and affordable method of diagnosis is currently unavailable. The etiologic agent of Lyme disease, *Borrelia burgdorferi*, is transmitted by the black-legged tick, *Ixodes scapularis*, whereas the bacterium that causes STARI, *B. lonestari*, is transmitted by the lone star tick, *Amblyomma americanum*. The American dog tick, *Dermacentor variabilis*, is also common in the eastern United States and is known to acquire but not transmit *B. burgdorferi*. Forest patch size has been shown to affect density of the white-footed mouse, *Peromyscus leucopus*, and is an important predictor of Lyme disease risk to humans because *P. leucopus* is the most efficient and abundant reservoir host for *B. burgdorferi*. The mouse is often more abundant in smaller forest patches. One objective of this research was to compare tick burdens of white-footed mice (*Peromyscus leucopus*), and the minimum infection rates (MIRs) of ticks by *B. burgdorferi* and *B. lonestari*, between small (<3 ha) and large (>3 ha) forest patches in Fredericksburg and Spotsylvania County Memorial National Military Park, located in Caroline, Orange, Spotsylvania, and Stafford Counties in Virginia. Neither *D. variabilis* nor *I. scapularis* have been tested for the ability to acquire *B. lonestari*, which was another objective of

this research. Mice were sampled randomly using Sherman live traps deployed in a grid design in forest patches. All ticks attached to the heads of mice were removed with forceps and preserved in 70% ethanol for laboratory analysis [DNA isolation and amplification, polymerase chain reaction (PCR), and nucleotide sequencing]. Ticks also were sampled by dragging a 1-m² white cloth through leaf litter and vegetation to determine densities. All adult and nymphal ticks and a sample of larval ticks found on the cloth were collected and preserved in 70% ethanol for laboratory analysis. Mouse density was significantly greater ($P < 0.05$) in small patches, but tick burdens showed no significant ($P > 0.05$) relationship with mouse density. No *A. americanum* were found on mice. *I. scapularis* and *D. variabilis* burdens and infestation rates did not differ significantly ($P > 0.05$) with patch size. Inadequate sample sizes of ticks within individual sites precluded the ability to compare infection rates between patch sizes. Juvenile *I. scapularis* and *D. variabilis* showed no selection for mice by age, sex, or weight. From drag samples densities of *I. scapularis* nymphs, nymphs and adults of *A. americanum*, and adult *D. variabilis* did not differ significantly ($P > 0.05$) by patch size. No functional dependence ($P > 0.05$) was found between mouse densities and densities of *A. americanum* larvae, nymphs, or adults, *D. variabilis* adults, or *I. scapularis* nymphs. Leaf-litter depth, ambient temperature, relative humidity, soil moisture, deer usage, and volume of coarse woody debris did not differ with size of forest patch and thus may be more important in determining tick burdens and tick densities than canopy cover, ground cover, or soil moisture, which did differ significantly ($P < 0.05$) by patch size. Neither patch size nor other habitat characteristics should be considered exclusive determinants of local tick burdens, MIRs, or risk of Lyme disease to humans. No *B. lonestari* was

detected by PCR analysis in larvae, nymphs, or adults of *D. variabilis*, larvae or nymphs of *I. scapularis*, or larval *A. americanum*. PCR results revealed a MIR of *B. lonestari* in *A. americanum* of 3.4% (8/237) in nymphs and 5.9% (1/17) in adults. No *B. burgdorferi* was detected in any tick species of any life stage collected, supporting the hypothesis that most cases of Lyme disease reported in the South are actually cases of STARI.

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INTRODUCTION

Lyme disease.—The incidence of Lyme disease, or Lyme borreliosis, has been on the rise since the Centers for Disease Control and Prevention (CDC) began monitoring in 1982 what is now the most common vector-borne infectious disease in the United States, and possibly the world (CDC 2001h; CDC 2004). Since monitoring began in the United States, cases of Lyme disease have been reported in Washington, D.C., and every state except Montana (CDC 2001c, 2001d, 2004), but the disease is focused in the northeastern and northcentral portions of the country (Fig. 1) where approximately 95% of reported cases occur (CDC 2004; Dennis et al. 1998). The etiologic agent of Lyme disease, the spirochetal bacterium, *Borrelia burgdorferi*, is maintained in an enzootic cycle in nature and is transmitted by vector ticks of the *I. ricinus* complex to avian, reptilian, and mammalian hosts (Burgdorfer et al. 1982; CDC 2001d; Mather 1993; Ostfeld and Keesing 2000b; Steere et al. 1977).

The most characteristic symptom of the ailment is a migrating circular rash, with or without a central clearing, which occurs in 60-80% of patients at the site of a bite by a tick of the *Ixodes ricinus* complex (CDC 2001g; Lane et al. 1991; Kamradt 2002; Steere 2001; Shapiro and Gerber 2000; VDH 1997). First recognized in Europe in 1909 by Swedish dermatologist Arvid Afzelius (Burgdorfer and Keirans 1983; Kamradt 2002; Spielman et al. 1993), the distinctive rash was initially reported in the United States from

Wisconsin in 1970 (Scrimanti 1970). However, Lyme disease was not described as a clinical entity until 1977 after the affliction of clusters of children with arthritis in and around Lyme, Connecticut, in 1975 led to extensive clinical investigations (CDC 2001e; Steere et al. 1977).

Symptoms of Lyme disease often become more disabling if allowed to progress. The rash, erythema migrans (EM), is the most characteristic symptom of infection and is often the 1st to arise, occurring in approximately 60-80% of those who contract Lyme disease within 1 month of inoculation but typically within 1-2 weeks (CDC 2001b, 2004; VDH 1997). Whether or not EM appears, other initial symptoms are mild and include headache, fever, fatigue, malaise, myalgia, and arthralgia (CDC 2001b, 2004). These symptoms usually disappear with antibiotic treatment with either doxycycline or amoxicillin (CDC 2004; VHD 1997), but they also can disappear without treatment. If untreated or if treatment fails, early signs of bacterial dissemination usually follow 1-4 months after inoculation (CDC 2004). These include secondary EM lesions, Bell's palsy, meningitis, encephalitis, and radiculopathy (CDC 2001b, 2004; Steere 2001). Treatment with ceftriaxone usually cures symptoms, but they also can disappear if untreated (CDC 2004; Steere 2001; VHD 1997). Late signs of bacterial dissemination occur 3-4 months after inoculation and include the more serious and often severe symptoms of headache, fatigue, arthritis (especially in large, weight-bearing joints), encephalitis, cognitive disorders, personality disorders, and neurological and cardiac problems (CDC 2001b, 2004; Steere 2001). With or without treatment with intravenous ceftriaxone or penicillin, these symptoms can become severe, chronic, and disabling, but the affliction is rarely fatal (CDC 2001b, 2004; Steere 2001).

Etiologic Agent

Of at least 12 species of *Borrelia*, only 3 are known to cause Lyme disease in humans; *B. garinii*, *B. afzelii*, and *B. burgdorferi*, but only *B. burgdorferi* occurs in the United States (CDC 2001a, 2001h; Kamradt 2002; Lin et al. 2002; Oliver 1996; Oliver et al. 2003; Richter et al. 2004). Although the clinical manifestations of Lyme disease are similar regardless of the species responsible, certain symptoms are more strongly associated with different species of *Borrelia* (CDC 2001a; Kamradt 2002; Lin et al. 2002; Oliver 1996). Cutaneous manifestations are more common with *B. afzelii* infection, neurological manifestations with *B. garinii*, and arthritis with *B. burgdorferi* (CDC 2001a; Lin et al. 2002).

Borrelia burgdorferi is a spirochetal bacterium 1st isolated from the midgut of *I. scapularis* (= *I. dammini*) in 1981 by Burgdorfer et al. (1982) and described in 1984 by Johnson et al. (1984). It is cultured in Barbour-Stoenner-Kelly (BSK) medium with replication requiring 12-24 hours (CDC 2001a). However, continuous culturing can result in a population quite different from the original (CDC 2001a). *B. burgdorferi* (reference strain B31) is unique in that it contains not only 17-20 linear and circular plasmids of a combined total of at least 533,000 base pairs (bp) but also a linear chromosome containing 910,725 bp comprising 853 coding genes (Fraser et al. 1997). Most genes on the plasmids are biologically nonfunctional (Fraser et al. 1997). The outer surface proteins (Osps) that are anchored to the outer membrane of *B. burgdorferi* are believed to be expressed differently in different hosts (Schwan et al. 1995), activate the immune system of the host (Brightbill et al. 1999), and regulate replication and subsequent invasion of the bacterium into the host (Ohnishi et al. 2001). Upon ingestion

of the blood meal by the tick most spirochetes change expression of their Osps, triggered by changes in temperature (Schwan and Piesman 2000; Schwan et al. 1995), cell density (de Silva et al. 1999; Schwan and Piesman 2000), growth phase (Ramamoorthy and Philipp 1998), and pH (Carroll et al. 1999, 2000). Spirochetes present in the tick prior to feeding express OspA, which binds them to the midgut epithelium of the tick. Once feeding begins, expression of OspA ceases and that of OspC ensues (Schwan and Piesman 2000; Schwan et al. 1995). With OspA no longer binding the spirochetes to the epithelium, OspC induces massive replication and aids in migration of the bacteria out of the midgut into the salivary glands and into the host (de Silva and Fikrig 1995; Ohnishi et al. 2001; Schwan and Piesman 2002). The presence of OspC triggers the immune system of the host, but its heterogeneity allows it to change its sequences to escape immunological pressure (Lin et al. 2002).

Vectors

In the United States the 2 vector species directly responsible for human contraction of Lyme disease are the western black-legged tick, *I. pacificus*, west of the Rocky Mountains, and the northern black-legged tick (deer tick), *I. scapularis* (*I. dammini*—Fig. 2), to the east (CDC 2001e; Lane et al. 1991; Oliver et al. 1993a, 1993b; Piesman and Happ 1997; Spielman et al. 1979). Other ixodid species (e.g., *I. neotomae*) that do not bite humans help maintain the zoonotic cycle by feeding on the same hosts as the human-biting vectors, thereby infecting the hosts upon which *I. scapularis* and *I. pacificus* feed (Lane et al. 1991; Oliver et al. 2003). The latter species are therefore known as “bridge vectors” because they form the bridge upon which the bacteria cross from nonhuman-biting carriers to humans. Otherwise, the disease generally would be

contained to rodent hosts with no passage to humans. Because a vector must be efficient at acquiring the bacterium and maintaining and transmitting it, *I. scapularis* is considered the only competent vector in the east (Mukolwe et al. 1992; Piesman and Happ 1997; Piesman and Sinsky 1988). Mukolwe et al. (1992) found that neither the American dog tick (*Dermacentor variabilis*) nor the lone star tick (*Amblyomma americanum*), both very common in the eastern United States, acquired *B. burgdorferi* whereas *I. scapularis* both acquired and successfully transmitted the bacterium. Piesman and Sinsky (1988) also found *I. scapularis* to be very efficient at acquiring, transstadially maintaining, and transmitting the pathogen, but in contrast to Mukolwe et al. (1992) they also documented acquisition of *B. burgdorferi* by both *D. variabilis* and *A. americanum*. However, neither of the latter species was successful at maintaining the bacterium, making them inefficient vectors. Similarly, Piesman and Happ (1997) documented the proficiency of *I. scapularis* as a vector for *B. burgdorferi* and found *D. variabilis* to acquire but not maintain the bacterium, but *A. americanum* did not acquire the pathogen in their study. Even with differing results, all 3 studies support the efficiency of *I. scapularis* and the inefficiencies of both *D. variabilis* and *A. americanum* as vectors of the Lyme disease bacterium.

The 2-year life cycle of *I. scapularis* (Fig. 3) consists of 4 life stages: the egg, larva, nymph, and adult (ALDF 2006; Fish 1993; Ostfeld 1997). Masses of up to 3,000 eggs (Gage et al. 1995; Oliver et al. 1993a; Ostfeld 1997) are laid in leaf-litter in the early spring and hatch into larvae in midsummer (ALDF 2006; Fish 1993; Van Buskirk and Ostfeld 1995). Larvae begin questing for such small mammal hosts as mice and chipmunks, and only those successful in obtaining blood meals will molt to the nymph

stage that begins host-seeking the following late spring/early summer (ALDF 2006; Burgdorfer and Keirans 1983; Fish 1993; Ginsberg et al. 2004; Ostfeld 2002; Van Buskirk and Ostfeld 1995). Nymphs that obtain blood meals molt into adults that lay eggs, provided a blood meal is obtained, and die the next spring (ALDF 2006; Fish 1993; Ginsberg et al. 2004; Ostfeld 1997, 2002). Infection with *B. burgdorferi* is maintained through all molts (Fish 1993; Ostfeld and Keesing 2000a, 2000b).

The nymph is the stage most responsible for maintaining the enzootic cycle and for infecting humans with Lyme disease (Fish 1993; Lane et al. 1991; Van Buskirk and Ostfeld 1995). Unlike *I. pacificus* nymphs (Lane et al. 1991; Mather 1993), *I. scapularis* nymphs are active earlier in the year than larvae (Barbour and Fish 1993; Fish 1993; Gage et al. 1995; Lane et al. 1991). Therefore, nymphs, which already have had a chance to become infected from blood meals (Fish 1993; Gage et al. 1995; Lane et al. 1991), feed on and potentially infect new hosts before uninfected larvae feed, thereby increasing the probability that a larva will become infected from its host. In addition to maintaining the cycle of infection in nature, the seasonality of nymph questing is also responsible for the majority of human Lyme disease cases. Nymphs seek hosts during the summer when human outdoor activity is at its peak (Barbour and Fish 1993). Not only do nymphs encounter the greatest number of humans during this period but they also are small enough to escape detection (Barbour and Fish 1993; Fish 1993; Ostfeld 1997). Larvae are even smaller than nymphs, but 99% hatch free of infection, so larvae that acquire their 1st blood meals undetected on humans most likely will not harbor the bacterium (Fish 1993; Piesman et al. 1986). Adults, which have had 2 chances to acquire infection (Fish 1993), are most active when human activity is reduced and are large enough to be

easily noticed and removed prior to the 24-36 hours after which probability of transmission increases (CDC 2001f; Fish 1993; Piesman et al. 1987). In areas of high Lyme disease incidence up to 35% of nymphs and 70% of adult *I. scapularis* can harbor the spirochete (LoGiudice et al. 2003; Ostfeld 1997).

All stages of *I. scapularis* exhibit spatial clumping due to restricted mobility, so dispersal of the vector is highly dependent upon host movements (Daniels and Fish 1990; Falco and Fish 1991; Ostfeld 1997; Ostfeld et al. 1995, 1996a; Stafford 1992; Telford et al. 1992; Van Buskirk and Ostfeld 1998). As a result of questing on or very near the ground, larvae and nymphs typically encounter small hosts like rodents and insectivores, whereas adults climb as high up as 1 m on vegetation to await the passage of larger hosts, usually white-tailed deer (*Odocoileus virginianus*), on which they feed and mate (CDC 2001d; Ostfeld 1997, 2002; Solberg et al. 2003; Spielman et al. 1985). Because deer move and disperse greater distances than rodents and insectivores, the greatest distance of vector dispersal is expected from adults that are attached to or mating on deer (Van Buskirk and Ostfeld 1998). However, immature *I. scapularis* are known to parasitize birds, which have the potential to move much greater distances than deer (Magnarelli et al. 1992; Ostfeld and Keesing 2000b; Van Buskirk and Ostfeld 1998). Therefore, the spread of infection or its local reduction depends greatly upon the taxa parasitized. However, other host attributes are more directly responsible for local rates of infection by *B. burgdorferi*.

Host Associations

Infection rates of the Lyme disease spirochete are high in the northeastern United States and low in other portions of the country because of host competence and host

diversity. Host or reservoir competence is the ability of a species to acquire, maintain, and transmit the pathogen to the vector (Lane et al. 1991; Mather 1993). To serve as an efficient reservoir for *B. burgdorferi* a host species must 1) be locally abundant, 2) have many locally infected individuals that serve as hosts to many juvenile vector ticks, 3) not become resistant to repeated tick feeding, 4) be easily infected and maintain the infection for long periods of time, possibly life, and 5) have limited home ranges that result in continued residence in tick-infested areas (Oliver et al. 2003; Spielman et al. 1993). These characteristics are exemplified by the white-footed mouse (*Peromyscus leucopus*), considered the primary reservoir for *B. burgdorferi* in the northeastern United States (Clark et al. 2001; Donahue et al. 1987; Levine et al. 1985; Mather 1993; Ostfeld 2002; Ostfeld and Keesing 2000a, 2000b; Spielman et al. 1985; Telford et al. 1990).

The home range of *P. leucopus* is 0.1-0.6 ha but varies seasonally and with habitat, food supply, population density, age, sex, and reproductive condition (Lackey et al. 1985; Linzey 1998). *P. leucopus* exhibits spontaneous ovulation and generally produces several litters per year, with generally 3-5 offspring per litter. Gestation is about 22-23 days, and young reach sexual maturity after approximately 6 weeks. Generally, the breeding season is bimodal, with peaks in the spring and late summer/early fall. White-footed mice are extreme habitat and dietary generalists, which explains the ability of the species to occupy habitats that differ considerably in quality (Lackey et al. 1985; Linzey 1998; LoGiudice et al. 2003). The species is omnivorous with diets of insects, insect larvae, arthropods, mast seeds, grass seeds, fruits, berries, buds, fungi, vegetation, and cultivated foods (Jones et al. 1998; Lackey et al. 1985; Linzey 1998). *P. leucopus* is generally more abundant in deciduous forests and brushy fields but because

of competitive exclusion and food and space limitations will disperse into coniferous forests and suboptimal fields with less canopy cover and woody debris (Ostfeld et al. 1995; Lackey et al. 1985; Linzey 1998).

Many other small mammals, such as the eastern chipmunk (*Tamias striatus*), house mouse (*Mus musculus*), northern short-tailed shrew (*Blarina brevicauda*), eastern gray squirrel (*Sciurus carolinensis*), meadow vole (*Microtus pennsylvanicus*), and Norway rat (*Rattus norvegicus*), have demonstrated reservoir competence, but not to the extent of *P. leucopus* because they lack at least 1, often many, of the requirements of competence (Craine et al. 1997; Godsey et al. 1987; Markowski et al. 1998; Matuschka et al. 1997; McLean et al. 1993; Slajchert et al. 1997; Telford et al. 1990). Many larger mammals, including white-tailed deer (Mather 1993; Ostfeld and Keesing 2000b; Telford et al. 1988), the raccoon (*Procyon lotor*—Godsey et al. 1987; Mather 1993), and the Virginia opossum (*Didelphis virginiana*—Godsey et al. 1987), are inefficient reservoirs for *B. burgdorferi*. However, the reservoir incompetence of these species is largely inconsequential because they host mainly adult ticks, the females of which produce infection-free larvae regardless of their own infection and then die (ALDF 2006; Fish 1993; Ostfeld 1997, 2002; Piesman et al. 1986). The tick life cycle ends with the adult and begins again with the larva.

In concert with host competence, host diversity plays a large role in determining Lyme disease prevalence. A greater diversity of hosts in a community is associated with a greater probability that a vector will feed on a species that is less competent than *P. leucopus*, thereby siphoning tick meals away from the most competent host, a concept called the “dilution effect” (Ostfeld and Keesing 2000a, 2000b). However, those same

hosts also can have the ability, depending on their reservoir competence, to maintain the spirochete within the community during a population decline in *P. leucopus*, a concept called the “rescue effect”. *I. scapularis* in the South and *I. pacificus* in the West feed predominantly on reservoir-incompetent lizards and less often on reservoir-competent rodents and insectivores (Apperson et al. 1993; Casher et al. 2002; Eisen et al. 2004; Lane 1990; Lane et al. 1991; Lane and Quistad 1998; Levin et al. 1996, Levine et al. 1997; Oliver et al. 1993a; Wright et al. 1998). The composition of the local host community and competence of its species are vital in maintaining prevalence of *B. burgdorferi*.

Because Lyme disease is detrimental to humans, it is rational to question whether it also is harmful to its other hosts. Viitala et al. (1986) found that infestations by ixodid ticks increased mortality significantly in gray-sided voles (*Clethrionomys rufocanus*), and Burgess et al. (1990) and Anderson and Magnarelli (1984) witnessed symptom manifestations in 16 of 195 and 5 of 122 wild-caught *P. leucopus*, respectively. Symptoms in the latter studies, confirmed for *B. burgdorferi* infection, ranged from EM lesions to severe and debilitating neurological damage. A few mice died and others were euthanized when they began exhibiting symptoms. Symptoms might not have been observed had the animals not been captured; the authors cautioned that they did not know what effect captivity had on expression of symptoms. Other researchers found *B. burgdorferi* infection but no evidence of its expression or increased mortality in wild-caught *P. leucopus* (Levine et al. 1985), red-backed voles (*C. gapperi*—Bey et al. 1995), or meadow voles (Campbell et al. 1994). The majority of research indicates that any adverse behavioral effects of Lyme disease in rodents that would decrease survival in the

wild are rare. It generally is accepted that Lyme disease in small mammal hosts has an insignificant impact on population dynamics (Gage et al. 1995; Ostfeld et al. 1996b).

Importance of Habitat and Microclimate

Habitat and microclimate variables contribute greatly to the distribution of the bacterium, its vector, and its hosts. The location of larvae in the summer is determined primarily by the location of deer in the fall, because adult females drop off deer and lay eggs where they fall (Maupin et al. 1991; Ostfeld 1997, 2002; Ostfeld et al. 1995, 1996a). The location of deer is greatly affected by the mast production of oak trees, which varies annually. In years of heavy production deer, with mating, adult *I. scapularis* attached, spend more time in these oak forests where they enjoy their preferred diet of acorns (McShea and Schwede 1993; Ostfeld et al. 1995, 1996a). In years of poor production deer spend more time in other habitats, like maple forests. As a result, larvae are expected to be most abundant in oak forests the year after heavy mast production, and nymphs are expected to be most abundant 2 years after, given the 1-year lag in life stage (Maupin et al. 1991; Ostfeld 1997; Ostfeld et al. 1995, 1996a). A year after poor production or mast failure, when deer are expected to forage elsewhere (McShea and Schwede 1993), larvae are expected to be less abundant in oak stands and more abundant in maple forests (Ostfeld 1997; Ostfeld et al. 1996a).

Masting events attract not only deer but also white-footed mice (Ostfeld 1997; Ostfeld et al. 1996a). Acorn abundance induces winter breeding behavior in *P. leucopus* where winter breeding does not generally occur (Jones et al. 1998). Mice consume and store acorns in great quantities, increasing their physiological condition and leading to increased survival rates and reproduction. The increased physiological condition and

consequent breeding of mice in winter leads to increased densities as the spring breeding season begins (Jones et al. 1998; Ostfeld et al. 1996a). Heavy mastings increase densities of both the primary vector for Lyme disease and its most competent reservoir host the following year. Therefore acorn production helps regulate local *B. burgdorferi* prevalence in a positive feedback loop that maintains the cycle of infection.

Several microclimate variables are associated with the ability of *I. scapularis* to survive, develop, expand its range, and transmit the Lyme disease spirochete. Ticks of the *I. ricinus* complex can tolerate temperatures from -10°C to 35°C with only brief fluctuations outside this range (Lane et al. 1991), but ambient temperatures above 27°C negatively affect spirochete survival within the tick (Shih et al. 1995). Relative humidity of at least 80% and near saturation of the soil are required for successful establishment of *I. scapularis* (Lane et al. 1991). However, too much moisture can enhance the growth of fungi and nematodes that feed on *I. scapularis* and consequently increase mortality (Guerra et al. 2002). High altitudes can restrict survival of *I. scapularis* by increasing desiccation (Lane et al. 1991). In addition to these extrinsic requirements, abundance of *I. scapularis* has been associated with environments with at least 50% canopy cover (Lubelczyl et al. 2004), abundant leaf-litter for overwintering protection (Guerra et al. 2002; Schulze et al. 1998), woody vegetation (Alder et al. 1992; Bertrand and Wilson 1996, 1997), and shrub cover (Schulze et al. 1998). Consequently, temperate deciduous forests are ideal for the establishment, survival, and reproduction of *I. scapularis*.

According to a habitat suitability model created by Brownstein et al. (2003), *I. scapularis* has the potential to expand its range in Virginia. Laboratory studies have shown 20-100% of *I. scapularis* to be infected with *B. burgdorferi* in the northeastern and

northcentral portions of the country compared to 4% in Virginia (VDH 1997). However, as of 2001 Lyme disease had been reported in 3 of the 4 counties in which FRSP is located (VDH 2001). One case has been documented in Caroline County, 1 in Stafford County, 2 in Spotsylvania County, but none in Orange County (VDH 2001).

Forest Fragmentation

Although *I. scapularis*, its major hosts, and the Lyme disease bacterium thrive in deciduous forests, controversy over the effect of forest patch size on the abundance of *I. scapularis* and prevalence of *B. burgdorferi* persists (Allan et al. 2002; Wilder and Meikle 2004). The theory of island biogeography (MacArthur and Wilson 1967) predicts that small, isolated islands will support less vertebrate diversity than mainland habitats. Many studies seem to support the theory and its species-area relationship when applied to habitat patches in the current highly fragmented terrestrial landscape (Anderson et al. 2003; Nupp and Swihart 1996, 1998, 2000; Rosenblatt et al. 1999). However, patch area alone cannot fully explain the causes of reduced species diversity of small mammals. Fragmentation causes not only changes in the total area of the patch but also increases the edge-to-interior habitat ratio, which can alter both the faunal and floral species composition within the patch and predatory and competitive pressures upon small mammals (Bender et al. 1998; Debinski and Holt 2000; Frank et al. 1998; Mather 1993; Nupp and Swihart 1996, 1998, 2000; Ostfeld 2002; Ostfeld and Keesing 2000b).

Although habitat destruction causes direct loss of habitat, fragmentation may or may not result in a large loss of area but will divide the contiguous landscape into isolated or connected patches surrounded by unsuitable habitat, creating a metapopulation (Bender et al. 1998; Debinski and Holt 2000; Hanski and Gilpin 2007; Ostfeld 2002;

Ostfeld and Keesing 2000b). Even small divisions that result in little habitat loss in the landscape can be sufficient to prevent dispersal through an inhospitable matrix for some species, which can lead to crowding of small mammals within the patch because of reduced emigration and dispersal (Debinski and Holt 2000). The presence of corridors between patches can promote dispersal, reducing crowding within the patch (Bender et al. 1998; Debinski and Holt 2000). A review of habitat fragmentation studies showed that fragmentation reduced species richness of small mammals in some cases, as predicted, but increased it in others (Debinski and Holt 2000). The results of the increased species richness were attributed to edge effects, which attracted species from surrounding habitats.

Forest edge is differentially exposed to such abiotic variables as wind and temperature (Bender et al. 1998; Debinski and Holt 2000). Edge habitat consequently contains more plant diversity, is structurally more complex than surrounding or interior habitats, and therefore supports more mammalian species, particularly generalists (Bender et al. 1998; Debinski and Holt 2000; Mather 1993; Robinson et al. 1992). When a forest becomes fragmented into patches the core area is reduced and the amount of edge surrounding the newly formed patches is increased. Thus fragmentation should have 1) the greatest negative impact on diversity of interior species, 2) the greatest positive impact on diversity of edge species, and 3) little or no effect on generalist species like *P. leucopus* (Bender et al. 1998; Ostfeld 2002).

Numerous studies have demonstrated the results of habitat fragmentation in forest patches of differing sizes (Anderson et al. 2003; Nupp and Swihart 1996, 1998, 2000; Robinson et al. 1992; Rosenblatt et al. 1999). All of these investigations found lower

mammalian diversity and greater abundance of *P. leucopus* in small compared to large forest patches. Investigators attributed the cause to the effects of increased edge-to-interior ratio. In addition to the edge effects described above, the authors argued that fragmentation leads to the release of both competitive and predatory pressures on *P. leucopus*. Reduced patch size generally hinders the abundance of such predators as owls and foxes, and species that compete with mice such as squirrels and chipmunks prefer more contiguous forests. This is possibly because larger forests can accommodate larger home ranges required by many competitors. Large forests also provide continuous habitat cover for these mouse competitors, preventing them from having to cross through a more open and unsuitable habitat to reach the next forest patch. Fragmentation would expose mice to predators of open spaces, such as hawks, and other dangers, such as crossing roads (Nupp and Swihart 1996, 1998; Ostfeld 2002; Ostfeld and Keesing 2000b). Generalists like *P. leucopus* thrive in the diverse edge habitats of the small patches, unimpeded by competition and predation.

With the abundance of *P. leucopus* well-documented in small forest patches, only 2 studies have examined the prevalence of *I. scapularis* or *B. burgdorferi* in forest patches of different sizes. Allan et al. (2002) found by drag sampling that 3 ecological indicators of Lyme disease risk, the density of nymphs (DON), the nymphal infection prevalence (NIP), and the density of infected nymphs (DIN) were correlated inversely with forest patch area. The density of larvae (DOL), however, was not significantly associated with patch size, suggesting that factors other than forest patch area contribute to the distribution of white-tailed deer. Two such possible factors are the large home ranges of deer and the interannual variation in mast production, which has been shown to

affect deer movement (McShea and Schwede 1993). Allan et al. (2002) concluded that the risk of contracting Lyme disease is higher in forest patches of ≤ 2 ha compared to larger patches because elevated DON, NIP, and DIN are combined with high densities of the primary reservoir with little species diversity to dilute infectious blood meals.

Wilder and Meikle (2004), however, did not test for infection rates of *B. burgdorferi* but examined tick infestations of mice within small and large forest patches. Their results seem to contradict the conclusion drawn by Allan et al. (2002). Wilder and Meikle found lower infestation of adult mice in smaller forest patches, suggesting that the risk of contracting Lyme disease is not elevated in smaller forest patches. However, there are numerous differences between the studies that might contribute to the apparently contradictory findings. The number of patches studied and the range of sizes differed considerably, as did the agricultural histories of the landscapes and matrices. Also, sites examined by Allan et al. were dominated by red maple, whereas those studied by Wilder and Meikle were extremely heterogeneous and not dominated by any single tree species. Finally, the latter did not quantify tick burdens but merely counted a mouse as infested or not based on the presence of a single juvenile tick.

Southern Tick-Associated Rash Illness.—Complicating the diagnosis of Lyme disease is an emerging illness called Southern Tick-Associated Rash Illness (STARI), or Master's disease, whose symptoms are indistinguishable from those of Lyme disease. STARI patients experience the same nonspecific symptoms as Lyme disease but also often exhibit the characteristic EM rash, all of which respond to antibiotic treatment (Campbell et al. 1995; Felz et al. 1999; Kirkland et al. 1997; Masters et al. 1998). EM rashes have been associated with the bites of *A. americanum* (Fig. 2) in the southern

United States since 1984 (Schulze et al. 1984). When all attempts to isolate *B. burgdorferi* from ticks and southern patients bitten by confirmed *A. americanum* failed, it was ruled out as the causative agent of the Lyme-like illness. However, because the Lyme-like symptoms vanished with antibiotic treatment, it was hypothesized that the causative agent was a different bacterium. The new illness initially was termed Master's disease after the Missouri physician, Dr. Edwin Masters, and colleagues who first suggested that the rashes were not caused by Lyme disease but by an unrecognized Lyme-like illness. Now, STARI is the more common reference to the illness. The cause of STARI was finally discovered in 1996 when molecular techniques revealed a newly discovered bacterium, *B. lonestari*, in *A. americanum* (Barbour et al. 1996). Since then, *B. lonestari* has been detected in *A. americanum* and hosts throughout the South (Armstrong et al. 2001; Bacon et al. 2003, 2005; Moore et al. 2003; Stegall-Faulk et al. 2003; Varela et al. 2004b) and from at least 1 human (James et al. 2001). The spirochete, which resists culture in the BSK medium suitable for *B. burgdorferi*, was finally cultured in a modified medium in 2004 (Varela et al. 2004a).

The geographic range of *A. americanum* stretches from central Texas northward to Missouri and eastward in a broad swath across the southern United States continuing northward in a thin band up the coast to Maine (Keirans and Lacombe 1998; CDC 2005; Fig. 4). This includes areas of overlap with the *B. burgdorferi*-vectoring *I. scapularis*, which raises questions about the diagnoses of Lyme disease patients in these areas of sympatry, especially in the South, given the identical symptoms of the 2 diseases, the lack of a reliable standard clinical test for either disease, and physician-related factors (James et al. 2001; Kirkland et al. 1997). Hundreds of supposed cases of Lyme disease

were misdiagnosed in Georgia in the 1980s, which led to more stringent diagnostic criteria (Barbour 1996; CDC 1990a, 1990b). Nevertheless, cases throughout the South continued to be misdiagnosed or diagnosed based on the presence of EM without serologic or other testing (Bacon et al. 2003, 2004; Barbour et al. 1996; Boltri et al. 2002; Oliver et al. 2000). The only irrefutable criterion for diagnosis is isolation of the spirochete from the patient, which typically is not attempted by physicians because of cost, difficulty, and impracticality (Oliver 1996).

Some even question whether Lyme disease actually occurs in the South (Barbour 1996) because of the rarity of human encounters with *I. scapularis* in that region (Armstrong et al. 1996, 2001; Felz et al. 1999; Merten and Durden 2000), the feeding behavior of *I. scapularis* in the South (Oliver 1996), and the lack of any isolates of *B. burgdorferi* from humans in the southern states (Barbour 1996; Kirkland et al. 1997; Oliver 1996; Piesman and Happ 1997). *A. americanum* is much more abundant in the South than *I. scapularis*, is a very aggressive and nonspecific feeder, and targets human hosts in all 3 mobile stages (Armstrong et al. 2001; Bishopp and Trembley 1945; Childs and Paddock 2003; James et al. 2001; Sonenshine 1979; Varela et al. 2004b). The species comprises the vast majority of tick attachments to and encounters by people in the South whereas *I. scapularis* is encountered only rarely (Armstrong et al. 1996, 2001; Felz et al. 1996; Kirkland et al. 1997). Thus any pathogen transmitted to humans in this region by ticks is likely transmitted by *A. americanum*, not *I. scapularis*. In the South *I. scapularis* is less likely to feed on hosts infected with *B. burgdorferi* because of the “dilution effect” created by the more diverse pool of hosts (Lane et al. 1991; Oliver 1996; Ostfeld and Keesing 2000a, 2000b). Southern *I. scapularis* feeds not only on a more

diverse mammalian host pool but predominantly on reservoir-incompetent lizards as opposed to its northern counterpart, which feeds heavily on the abundant and highly reservoir-competent *P. leucopus* (Apperson et al. 1993; Lane et al. 1991; Levine et al. 1997; Oliver 1996). In addition, *I. scapularis* nymphs in the South do not feed seasonally before larvae do and therefore do not inoculate hosts, so that larvae are less likely to acquire the bacterium (Oliver 1996). These are among the reasons why Lyme disease is focused in the northern United States. *B. burgdorferi* is well documented from ticks and wildlife throughout the southern states (those south of the Mason-Dixon line—Clark 2004; Felz et al. 1999; Merten and Durden 2000; Oliver 1996; Oliver et al. 2000, 2003; Piesman and Sinsky 1988), but Barbour (1996) argued that the presence of the pathogen does not guarantee transmission to humans. Although Lyme disease cases are reported each year in southern states, isolation of *B. burgdorferi* from a human in the South has not been documented.

The problem must now be addressed that 2 different but clinically similar diseases exist in parts of the United States. *B. lonestari* responds to antibiotics so a cure is not the problem, but much less is known about STARI, its etiologic agent, and its epidemiology because of its relatively new discovery compared to Lyme disease. We will not know the accurate number of Lyme disease and STARI cases in the United States until an affordable standard clinical test is developed and physicians, especially in the South, become more aware of STARI. Until then, Lyme disease may continue to be overdiagnosed and STARI underdiagnosed in the South and other areas of overlap in vector ranges.

Etiologic Agent

Like all members of its genus, *B. lonestari* is a spirochetal bacterium (Barbour et al. 1996; Varela et al. 2004a). Before its molecular discovery in 1996, the etiologic agent of STARI was distinguishable from *B. burgdorferi* only by its lack of cultivability in the BSK medium suitable for other *Borrelia* species (Barbour et al. 1996; Campbell et al. 1995; Kirkland et al. 1997; Masters et al. 1998; Oliver 1996). Microscopic examination of blood samples taken from EM-exhibiting patients bitten by confirmed *A. americanum* and from the ticks themselves revealed spirochetes that were assumed to be *B. burgdorferi*; however, all attempts to culture them in BSK media failed (Bacon et al. 2003; Barbour et al. 1996; Campbell et al. 1995; James et al. 2001; Kirkland et al. 1997; Masters et al. 1998; Oliver 1996). The consistent findings led to the suspicion of a new Lyme-like illness now known as STARI. Barbour et al. (1996) used *Borrelia* genus-specific primers to amplify 2 separate genes in the spirochetes to discover sequences distinct from other known species of *Borrelia* and named it *B. lonestari*. His further examination, which was later supported by Bacon et al. (2004) and Rich et al. (2001), revealed that *B. lonestari* is closer in relation to the relapsing fever group of spirochetes than to *B. burgdorferi*. The recent cultivation of *B. lonestari* (Varela et al. 2004a) will facilitate a greater understanding of its cellular structure, clinical manifestations, epidemiology, and vector and host competences.

To date, the average infection rate of known *B. lonestari* or uncultivable spirochetes (prior to the molecular method of distinction) in *A. americanum* throughout the southern United States is approximately 2% with researchers reporting a range of 1-5.6% (Armstrong et al. 2001; Bacon et al. 2003, 2005; Barbour et al. 1996; Clark 2004;

Varela et al. 2004b). Schulze et al. (2005) documented a prevalence of 9.1% in adult *A. americanum* in New Jersey and Moore et al. (2003) found infection in 8.7% of white-tailed deer examined.

Vector

Until the 1990s *A. americanum* was not regarded as a major vector of disease in humans or other animals but merely as a pest to livestock (Childs and Paddock 2003). It is now known as a vector for the emerging *B. lonestari* and many other pathogens, including *Ehrlichia chaffeensis*, the agent of human monocytic ehrlichiosis (HME), *E. ewingii* and *Anaplasma phagocytophila*, both agents of human granulocytic ehrlichiosis (HGE), *Francisella tularensis*, the agent of tularemia, *Coxiella burnetii*, the agent of Q fever, *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever, and other species of *Rickettsia* (Bishopp and Trembley 1945; Childs and Paddock 2003; Kollars et al. 2000; Stromdahl et al. 2000; Varela et al. 2004b). The explosive population growth of the white-tailed deer, which hosts all mobile stages of *A. americanum*, the expanding geographic range of the tick, its aggressive feeding behavior, and its broad range of hosts have contributed to the emergence of *A. americanum* as a major disease vector (Childs and Paddock 2003; Varela et al. 2004b).

The life cycle and ecological requirements of *A. americanum* are generally similar to those of *I. scapularis* (CDC 2005; Sonenshine 1979; Sonenshine and Levy 1971). The 4-stage life cycle requires up to 2 years to be completed with larvae hatching in late spring and early summer (Sonenshine and Levy 1971). Some newly hatched larvae feed, drop from the host, molt, and begin seeking hosts as nymphs during the same season. The authors state that unfed larvae rarely survive whereas the survival of unfed nymphs is

common. Nymphs that obtain blood meals molt to adults, which overwinter then feed and lay eggs the following spring (Sonenshine and Levy 1971).

Seasonal activity of the various life stages can vary slightly both spatially and temporally. All life stages of *A. americanum* have been found on hosts throughout the year in the southern United States but are less abundant in midwinter (Bishopp and Trembley 1945). Studies have documented that questing activity of adult *A. americanum* peaks in late May through early July in eastern Oklahoma (Patrick and Hair 1977), May in southeastern Missouri (Kollars et al. 2000), and May or June in Virginia (Sonenshine 1979; Sonenshine and Levy 1971). The greatest nymphal questing activity has been documented from March to October (with no distinctive peak) in eastern Oklahoma (Patrick and Hair 1977) and in June in southeastern Missouri (Kollars et al. 2000) and Virginia (Sonenshine 1979; Sonenshine and Levy 1971). Larval peaks have been documented from late July through early August in eastern Oklahoma (Patrick and Hair 1977), September in southeastern Missouri (Kollars et al. 2000), and August or September in Virginia (Sonenshine 1979; Sonenshine and Levy 1971). As with *I. scapularis*, questing of *A. americanum* nymphs before larvae increases the probability that larvae will acquire any pathogens from hosts possibly infected by nymphs earlier in the season (Childs and Paddock 2003).

Importance of Habitat and Microclimate

Temporal variation in questing activity of the various life stages of *A. americanum* has been documented from many southern states and is attributed to microclimate variables (Kollars et al. 2000; Patrick and Hair 1977). Kollars et al. (2000) cited several studies that reported slight variation in peak activity from one year to the

next and unimodal peaks some years and bimodal peaks in others. Patrick and Hair (1977) found twice as many adult female *A. americanum* infesting white-tailed deer in 1973 than in 1974. The authors recorded significantly less moisture and humidity in 1974 and suggested that this deficit likely forced ticks to remain in the moist cover of the leaf-litter instead of exposing themselves to the desiccating environment to seek hosts. Also, deer were approximately 20% less abundant in 1973, which would have limited tick reproduction on this host. As with *I. scapularis*, the abundance of adult *A. americanum* in a given year is affected by the abundance of nymphs the preceding year, which is affected by the abundance of larvae the year before that (Sonenshine and Levy 1971). Many ecologically linked variables that differ annually, like precipitation, acorn production, and host abundance, contribute to annual fluctuations in tick abundance.

Like *I. scapularis*, *A. americanum* is significantly less abundant inland than along the coast (Sonenshine and Levy 1971). Adult and nymphal densities of *A. americanum* at the time of peak activity were 4.4 and 2.3 times greater, respectively, in the coastal study area. *A. americanum* can be found in many different habitat types, including fields, but is most abundant in forests (Bishopp and Trembley 1945; Sonenshine and Levy 1971). Sonenshine and Levy (1971) found no consistent relationship between tick distribution and forest types.

Host Associations

Although *A. americanum* is rarely found on small mammals, the host range of this parasite is very broad and includes large and medium-sized wild and domestic mammals and birds, particularly ground dwellers (Bishopp and Trembley 1945; Kollars et al. 2000; Sonenshine 1979). The white-tailed deer is the most important large mammal host,

serving not only as the breeding ground for adults but as a vital source of blood meals for all mobile stages of the tick (Bishopp and Trembley 1945; Kollars et al. 2000; Patrick and Hair 1977). This host's resurgence is blamed for the rapid and efficient spread of diseases carried by *A. americanum* (Childs and Paddock 2003). Some researchers have reported astounding numbers of *A. americanum* covering deer (Bishopp and Trembley 1945; Bolte et al. 1970). Approximately 4,800 ticks, most of which were *A. americanum* nymphs, were reported from a count from 1 ear of a deer in South Carolina in 1937 (Bishopp and Trembley 1945). Infestations can be severe enough to cause detrimental effects on the health of hosts and even lead to death (Bishopp and Trembley 1945; Bolte et al. 1970; Ervin et al. 1987).

Many medium-sized mammals have been documented as hosts for *A. americanum* and include the raccoon, Virginia opossum, eastern gray squirrel, red fox (*Vulpes vulpes*), coyote (*Canis latrans*), and eastern cottontail rabbit (*S. floridanus*—Bishopp and Trembley 1945; Jacobson et al. 1978; Kollars et al. 2000). Data reported from several researchers regarding the importance of rabbits as hosts illustrate the geographic and even regional variation in host associations. The rabbit presumably is not an important host to *A. americanum* (Bishopp and Trembley 1945), and Sonenshine et al. (1965) report a lack of infestation in rabbits in their study sites in Virginia. However, Jacobson et al. (1978) found the host to be an important one for *A. americanum* in a different area of the same state. The rabbit also was reported as an important host in Tennessee (Zimmerman et al. 1988) and Missouri (Kollars et al 2000), where 1 rabbit was infested with 1,100 larvae.

Severe infestations of *A. americanum* on hosts can have detrimental and even fatal effects (Bishopp and Trembley 1945; Bolte et al. 1970; Ervin et al. 1987). Ervin et

al. (1987) attribute weight gain reduction in cattle to *A. americanum* infestation and Bishopp and Trembley (1945) believe that increases in wild turkey (*Meleagris gallopavo*) mortality may be attributed to gross infestations of these parasites. In a study of white-tailed deer fawns less than 6 weeks old, Bolte et al. (1970) found deaths or imminent deaths to be caused by tissue destruction, blood loss, necrosis, infection, and blindness caused by intensive feeding by *A. americanum* around the eyes of the animals. The authors estimated a 34% loss of deer fawns due to ticks for the year in which their research was conducted.

Because *A. americanum* was the suspected vector of STARI during its emergence, it has received all of the attention regarding *B. lonestari*. Currently, no other vectors of *B. lonestari* are recognized. *D. variabilis* (Fig. 2) is capable of acquiring the Lyme disease bacterium (Piesman and Happ 1997; Piesman and Sinsky 1988) but launches an antimicrobial defense that kills the bacteria upon inoculation (Johns et al. 2000). No published research to my knowledge has tested this known vector of Rocky Mountain Spotted Fever, tularemia, and tick paralysis (Bishopp and Trembley 1945; Sonenshine 1979) or *I. scapularis* for the ability to acquire the STARI bacterium.

Although only about 4% of *I. scapularis* harbor *B. burgdorferi* in Virginia, compared to 20-100% in endemic areas of the North (VDH 1997), the Virginia Department of Health (VDH) reports that the number of Lyme disease cases throughout the state continue to grow each year, with a 27% increase from 2004 to 2005 (VDH 2006). Perhaps this is support for the habitat expansion model of *I. scapularis* put forth by Brownstein et al. (2003). The VDH suggests that increased awareness by both the public and healthcare professionals, increased case follow-up, and increased urbanization

all contribute to the increased incidence and detection of the disease (VDH 2006). Also, cases of Rocky Mountain Spotted Fever (RMSF), harbored by *D. variabilis*, have increased steadily and spiked to a 169% increase from 2004 to 2005 (VDH 2006). Although ehrlichiosis, carried by *A. americanum*, is reported infrequently in Virginia, it also is on the rise (VDH 2006). The disease surveillance statistics of the VDH indicate the need to investigate the reasons for these trends. That VDH does not acknowledge STARI in its epidemiology reports indicates the need to investigate it. No studies have investigated tick densities, tick burdens, or Lyme disease or incidence of STARI within Fredericksburg and Spotsylvania County Memorial National Military Park. No density estimates for *I. scapularis*, *D. variabilis*, or *A. americanum* exist for the 4 counties that contain the park, but all 3 species are common to the region (Bishopp and Trembley 1945; CDC 2001e; Lane et al. 1991).

OBJECTIVES AND HYPOTHESES

The main objectives of this study were to 1) examine the relationship between forest patch size and *I. scapularis*, *A. americanum*, and *D. variabilis* burdens on small mammals, particularly *P. leucopus*, 2) examine the relationship of tick densities to forest patch size and season, 3) compare infection rates of the bacteria that cause Lyme disease (*B. burgdorferi*) and STARI (*B. lonestari*) between small (<3 ha) and large (>3 ha) forest patches, and 4) test 2 species (*D. variabilis* and *I. scapularis*) not yet examined as possible vectors of STARI to determine their abilities to acquire *B. lonestari* in Fredericksburg and Spotsylvania County Memorial National Military Park in northeastern Virginia. I hypothesized that tick burdens (all species) on *P. leucopus* would be greater in small patches because of lower mammalian diversity and higher densities of *P. leucopus* in the absence of competitors and predators. I hypothesized that if the incidence of *I. scapularis* and *A. americanum* burdens on *P. leucopus* was greater in smaller patches, MIRs of *B. burgdorferi* and *B. lonestari* also would be greater. I predicted that because *D. variabilis* has been shown to acquire [but not efficiently maintain] *B. burgdorferi*, this tick, and similarly, *I. scapularis*, would acquire *B. lonestari*. If these hypotheses were supported, the implications would be that the risk of contracting both Lyme disease and STARI is greater in small deciduous forest patches

compared to larger contiguous forests and that *D. variabilis*, *I. scapularis*, and other tick species deserve further attention as possible additional vectors for STARI.

METHODS AND MATERIALS

Study area.—Fredericksburg and Spotsylvania County Memorial National Military Park (FRSP—38°15'57"N, 77°28'38"W), established in 1927, is located in both the Appalachian Piedmont and Atlantic Coastal Plain physiographic provinces (FRSP 2005b; Linzey 1998). Elevation within the park ranges from near sea level to over 106 m (FRSP 2005a). Regional annual precipitation averages 102 cm, including snowfall (FRSP 2005c). With a mean annual temperature of 13° C, the park experiences summer highs of approximately 30° C and lows of 17°C and winter highs of approximately 9° C and lows of -4° C (FRSP 2005c).

FRSP is comprised of 9 units in Caroline, Orange, Spotsylvania, and Stafford counties. Of the 2,950 ha owned by the park, 492 ha are open fields, 378 ha are under cultivation (hay or row crops), and 2,457 ha are forested. Approximately 223 ha contain wetland features (Gregg Kneipp, pers. comm.), and *Castor canadensis* (American beaver) is documented in many of the wetland habitats (C. L. Tanner, pers. obs.).

Noncultivated fields consist of a variety of grasses (Gramineae) and sedges (Cyperaceae), poison ivy (*Toxicodendron radicans*), Virginia creeper (*Parthenocissus quinquefolia*), and tree saplings (oaks [*Quercus* spp.], hickories [*Carya* spp.], winged sumac [*Rhus copallina*], sweet gum [*Liquidambar styraciflua*], and tulip poplar [*Liriodendron tulipifera*])—C. L. Tanner, pers. obs.; NPSpecies 2006; Petrides 1986).

Management practices imposed upon fields include annual mowing and prescribed burning (Gregg Kneipp, pers. comm.). Mammals known to inhabit fields in the park are the northern short-tailed shrew, meadow vole, eastern harvest mouse (*Reithrodontomys humulis*), house mouse, meadow jumping mouse (*Zapus hudsonius*), and woodchuck (*Marmota monax*—Barry 2005; C. L. Tanner, pers. obs.; NPSpecies 2006).

The majority of forests are mixed deciduous with canopy species comprised of oaks, hickories, maples, sweet gum, and tulip poplar (C. L. Tanner, pers. obs.; NPSpecies 2006). The understory commonly contains poison ivy, late low blueberry (*Vaccinium angustifolium*), mountain laurel (*Kalmia latifolia*), cherry (*Prunus* spp.), and dogwoods (*Cornus* spp.—C. L. Tanner, pers. obs.; NPSpecies 2006; Petrides 1986). FRSP also harbors stands of short-leaf pine (*Pinus echinata*) and loblolly pine (*P. taeda*) and mixed deciduous/coniferous forests, containing the above understory species but also American holly (*Ilex opaca*—C. L. Tanner, pers. obs.; NPSpecies 2006; Petrides 1986). The most common forest-dwelling species found in the park are the white-footed mouse, white-tailed deer, raccoon, Virginia opossum, and gray squirrel. Other forest-dwelling species include the eastern chipmunk, northern short-tailed shrew, southeastern shrew (*Sorex longirostris*), least shrew (*Cryptotis parva*), pygmy shrew (*S. hoyi*), red fox, gray fox (*Urocyon cinereoargenteus*), and striped skunk (*Mephitis mephitis*—C. L. Tanner, pers. obs.; Linzey 1998).

Peromyscus leucopus is the most common small mammal captured in FRSP forests (C. L. Tanner, pers. obs.). Other small species known to occur in FRSP that have demonstrated host competence in various studies include the eastern chipmunk (Gage et al. 1995; Mather 1993; McLean et al. 1993; Ostfeld and Keesing 2000b; Slajchert et al.

1997), meadow vole (Anderson et al. 1986; Campbell et al. 1994; Markowski et al. 1998; Peavey et al. 1997), northern short-tailed shrew (Gage et al. 1995; Mather 1993; Ostfeld and Keesing 2000b; Telford et al. 1990), and gray squirrel (Craine et al. 1997; Gage et al. 1995; Godsey et al. 1987). The eastern chipmunk is scarce in the park, and the meadow vole is found nearly exclusively in fields. The northern short-tailed shrew and gray squirrel are commonly found in forested sites within the park (C. L. Tanner, pers. obs.).

Study site selection.—Forest patches were located according to size using aerial photos (Fig. 5). The habitat characterization and area of each patch were determined using a Geographic Information System (GIS) habitat layer and subsequently ground-truthed for verification of size and habitat type. I attempted to locate forest patches similar in habitat, but avoidance of the interannual impact of masting events was not possible because oaks are abundant in FRSP (Barry 2005; NPSpecies 2006). However, I proceeded under the assumption that the close proximity of all sites is sufficient to assume negligible regional variation. Three small (<3-ha) and 3 large (>3-ha) forest patches were chosen for replication, with a patch being defined as a forest fragment surrounded on ≥ 3 sides by nonforested habitat and, if on only 3 sides, bordered on the 4th side by a road or stream that may serve as a barrier to dispersal.

Habitat characterization.—Dominant tree species and canopy cover were used to establish habitat similarity. Habitat variables were quantified at 25 randomly chosen traps in large patches and 12 in small patches and averaged within each grid. General similarity among sites was determined by dominant tree species and percent canopy cover, obtained with a densiometer held directly over each randomly chosen trap and then averaged for the site. Percent ground cover was determined by eye calibration within 1

m² surrounding randomly chosen traps (i.e., with the trap in the center of the plot) and averaged for the site (Higgins et al. 1996). I defined ground cover as any vegetation, dead or alive, covering the ground, giving mice and ticks cover from above. Leaf-litter depth was determined with a ruler at each corner of a 1-m² plot surrounding each randomly chosen trap, averaged for each trap station, and then averaged to obtain a mean for the site. Volume (cm³/ha) of coarse woody debris (CWD) was estimated using a line-intercept method (Van Wagner 1982). Microclimate variables vital to the survival and development of *I. scapularis* also were recorded. Ambient temperature and relative humidity were measured daily during the morning trap checks with an Extech Instruments (Waltham, MA) digital hygro-thermometer (model 445702) placed on the ground at the microclimate that ticks experience. Soil pH and soil moisture also were measured daily at the centers of each grid with a Kelway Soil® Acidity and Moisture Tester (model HB-2—Kel Instruments Co., Inc., Wyckoff, NJ). Habitat and microclimate data were recorded from the same 6 sites during each trap session and each season. Seasons were defined by calendar dates; spring began 20 March, summer began 21 June, and fall began 23 September.

Tick collection.—Ticks were collected daily from small mammals and monthly by drag sampling, or flagging. Small mammal trapping is considered the most accurate sampling technique for estimating *I. scapularis* density in areas of low prevalence like Virginia (Lane et al. 1991; Solberg et al. 2003). All juvenile ticks were removed with forceps from each rodent and counted to determine tick burdens. All ticks were stored in 70% ethanol (Clark et al. 2001; Falco and Fish 1992; Ostfeld et al. 1996b) for later identification and lab determination of *Borrelia* spp. infection.

Tick densities within patches were determined through drag sampling, which consists of pulling a 1-m² white cloth along the ground, leaf litter, and vegetation (Falco and Fish 1992). The cloth was dragged along randomly chosen transects and checked every 20 m (Daniels et al. 2000; Falco and Fish 1992; Schulze and Jordan 2001). Ticks were counted, collected, and stored in 70% ethanol (Clark et al. 2001; Falco and Fish 1992; Ostfeld et al. 1996b) for later identification and lab determination of *Borrelia* spp. infection.

Mammal sampling.—Sherman live traps (7.6 x 8.9 x 22.9 cm) were arrayed in a grid configuration at each site from late April through mid-October 2005 to sample the nymphal and larval stages of *I. scapularis* on *P. leucopus*. The number of traps used depended on the size of the patch being sampled. Small patches contained 49 traps at 10-m intervals in 7 x 7 grids for an effective trapping area of approximately 1.12 ha. The boundary strip method (half the mean maximum distance moved [MMDM]) was used to determine the effective area of the grid (Krebs 1999; Otis et al. 1978; Parmenter et al. 2003; Wilson and Anderson 1985). The trapping effort in large patches was 100 traps at 10-m intervals in 10 x 10 grids to account for increased area, yielding an effective trapping area of approximate 3.28 ha. In larger patches 1 side of the grid was located along an edge of the forest patch and the rest of the grid extended into the interior of the patch to incorporate both edge and interior habitats. Although small patches inherently contain more edge than large patches because of the increased edge-to-interior ratio, traps in small patches were arrayed in the same configuration for consistency.

Traps were baited with a mixture of peanut butter, bird seed, and oats and were checked once per day. To create blocking effects, 1 small and 1 large grid were sampled

simultaneously for 2 or 3 consecutive days (the schedule was adjusted from 3 days in the beginning to 2 for the remainder of the study) approximately every 2 weeks from late April through mid-October. The 1st set of grids was followed by another set, which was followed by the last set, and the cycle continued again beginning with the 1st set of grids. Captured *P. leucopus* were examined for juvenile *I. scapularis*, which usually attach to the pinnae (Ostfeld et al. 1993). Species, age, gender, reproductive status, and weight of each mouse were recorded, and each individual was marked using the toe-clipping method (Rudran 1996) for individual identification. Pellet group surveys (pellet groups/m²) adapted from Mayle et al. (2000) and Wemmer et al. (1996) were conducted to estimate deer usage at each site.

Prevalence of *Borrelia* spp.—Several laboratory methods are available for diagnosing *B. burgdorferi* infection. Most studies conducted in high-prevalence regions use such methods as darkfield microscopy and direct and indirect immunofluorescent microscopy (Burgdorfer et al. 1982; Burgess et al. 1990; Oliver et al. 2003; Piesman and Happ 1997; Van Buskirk and Ostfeld 1998). However, in areas of low prevalence, like Virginia, polymerase chain reaction (PCR) is the recommended method for accuracy (Bacon et al. 2003; King 2004; Rosa and Schwann 1989; Rosa et al. 1991; Spielman et al. 1993).

DNA Isolation

Ticks were refrigerated at 4° C in 70% ethanol until isolation of DNA. All adult ticks, *I. scapularis* nymphs, and any ticks that were lone captures of its species and life stage from a drag session or host were processed individually, but most nymphs and larvae were pooled for extractions according to location, species, developmental stage,

and feeding status (questing, partially fed, or fully engorged). Each individual and pool was bisected with a sterile disposable 18-gauge NoKor needle. Bisections were conducted on a glass surface that was cleaned with 95% ethanol and flame-sterilized after each use. Extractions were performed using both an IsoQuick Nucleic Acid Extraction Kit (ORCA Research Inc., Bothell, Washington) and a Qiagen DNeasy Tissue Kit (Qiagen Inc., Valencia, California) according to the manufacturers' protocols. DNA isolates were stored at 4° C until PCR amplification.

DNA Amplification

As a positive control for *Borrelia* spp. isolation, an approximate 300-bp portion of the mitochondrial 16S rRNA tick gene, common to all tick taxa, was amplified using primers 16S+2 and 16S-1 (Black and Piesman 1994) to verify the presence of DNA. Reactions were carried out using puReTaq Ready-To-Go PCR Beads (GE Healthcare, formerly Amersham Biosciences, Piscataway, New Jersey) following the manufacturer's protocol. Each 25- μ l reaction contained 1 puReTaq™ Ready-To-Go™ PCR Bead (200 μ M of each dNTP in 10 mM Tris-HCL [pH 9.0 at room temperature], 50 mM KCl, 1.5 mM MgCl₂, and ~2.5 units of puReTaq DNA polymerase), purified water, 1 μ M of each primer, and approximately 50 ng/ μ l of template DNA. The thermal profile consisted of an initial denaturation at 94° C for 2 min followed by 30 cycles of denaturation at 94° C for 1 min, annealing at 50° C for 30 sec, and extension at 72° C for 1 min, a final extension at 72° C for 10 min, and an indefinite hold at 4° C (Black and Piesman 1994; Stromdahl et al. 2000, 2001). PCR products were electrophoresed on 2% agarose gels stained with ethidium bromide and visualized with an ultraviolet (UV) transilluminator.

The presence of a band at approximately 300 bp was accepted as verification of tick DNA. Each PCR run contained a sample of purified water as a negative control.

Borrelia spp. DNA was amplified by nested PCR using puReTaq Ready-To-Go PCR Beads (GE Healthcare, formerly Amersham Biosciences, Piscataway, New Jersey) according to the manufacturer's protocol with flagellin gene primers described by Barbour et al. (1996). Each 25- μ l reaction contained 1 puReTaq Ready-To-Go PCR Bead (200 μ M of each dNTP in 10 mM Tris-HCL [pH 9.0 at room temperature], 50 mM KCl, 1.5 mM MgCl₂, and ~2.5 units of puReTaq DNA polymerase), purified water, 1 μ M of each primer, and 0.682-1.429 ng/ μ l of template DNA. The external reaction used primers FlaRL and FlaLL to amplify an approximate 370-bp portion of the *Borrelia* genome. The internal reaction amplified an approximate 240-bp portion of the first product using primers FlaRS and FlaLS. All reactions contained a sample of verified *B. burgdorferi* DNA (a gift from Dr. Wirtz of the CDC, Atlanta, Georgia) and a sample of purified water as positive and negative controls, respectively. For both external and internal reactions the thermal profile consisted of an initial denaturation at 95° C for 2 min followed by 40 cycles of denaturation at 95° C for 1 min, annealing at 55° C for 1 min, and extension at 75° C for 1 min, a final extension at 75° C for 5 min, and an indefinite hold at 4° C (Barbour et al. 1996; Morozova et al. 2002; Stromdahl et al. 2003). PCR products were electrophoresed on 2% agarose gels stained with ethidium bromide and visualized with a UV transilluminator. Presence of bands at approximately 240 bp from internal reactions were considered positive for *Borrelia* spp. infection (Barbour et al. 1996).

PCR samples positive for *Borrelia* spp. were further analyzed by nested PCR using primers specific for each bacterium. Samples were amplified once using the p66 gene primers a, a', f, and f' described by Rosa et al. (1991) specific for *B. burgdorferi* and once using nested flagellin gene primers modified by Dr. Phillip C. Williamson (unpublished—DNA Identification Laboratory, Department of Pathology and Anatomy, University of North Texas Health Science Center, Fort Worth, Texas) to target *B. lonestari*. Reactions were carried out using puReTaq Ready-To-Go PCR Beads (GE Healthcare, formerly Amersham Biosciences, Piscataway, New Jersey) according to the manufacturer's protocol.

Both external and internal 25- μ l reactions using *B. burgdorferi*-specific primers contained 1 puReTaq Ready-To-Go PCR Bead (200 μ M of each dNTP in 10 mM Tris-HCL [pH 9.0 at room temperature], 50 mM KCl, 1.5 mM MgCl₂, and ~2.5 units of puReTaq DNA polymerase), purified water, 1 μ M of each primer, and 0.682-1.429 ng/ μ l of template DNA. External reactions used primers a and a', and internal reactions used primers f and f'. All reactions contained a sample of verified *B. burgdorferi* DNA (a gift from Dr. Wirtz of the CDC, Atlanta, Georgia) and a sample of purified water as positive and negative controls, respectively. For both external and internal reactions the thermal profile consisted of an initial denaturation at 94° C for 1 min followed by 30 cycles of denaturation at 94° C for 1 min, annealing at 50° C for 30 sec, and extension at 70° C for 1 min, a final extension at 70° C for 3 min, and an indefinite hold at 4° C (Rosa et al. 1991; Stromdahl et al. 2001). PCR products were electrophoresed on 2% agarose gels stained with ethidium bromide and visualized with a UV transilluminator. Presence of

bands at approximately 240 bp from internal reactions were considered positive for *B. burgdorferi* infection (Rosa et al. 1991).

Both external and internal 25- μ l reactions using *B. lonestari*-specific primers contained 1 puReTaq Ready-To-Go PCR Bead (200 μ M of each dNTP in 10 mM Tris-HCL [pH 9.0 at room temperature], 50 mM KCl, 1.5 mM MgCl₂, and ~2.5 units of puReTaq DNA polymerase), purified water, 1 μ M of each primer, and 0.682-1.429 ng/ μ l of template DNA. External reactions used modified primers FlaLL and FlaRL and internal reactions used modified primers FlaLS and FlaRS (C. P. Williamson, unpubl. data). All reactions contained a sample of verified *B. lonestari* DNA (a gift from Dr. Stromdahl of the USACHPPM, Aberdeen Proving Ground, Maryland) and a sample of purified water as positive and negative controls, respectively. For both external and internal reactions the thermal program consisted of an initial denaturation at 95° C for 3 min followed by 40 cycles of denaturation at 95° C for 1 min, annealing at 55° C for 1 min, and extension at 75° C for 1 min, a final extension at 75° C for 3 min, and an indefinite hold at 4° C (Barbour et al. 1996; Stromdahl et al. 2003). PCR products were electrophoresed on 2% agarose gels stained with ethidium bromide and visualized with a UV transilluminator. Presence of bands at approximately 240 bp from internal reactions were considered positive for *B. lonestari* infection (Barbour et al. 1996).

All PCR reactions were carried out using a PTC 200 DNA Engine (MJ Research). To reduce the chance of contamination, DNA isolations and PCR reactions were carried out in separate locations, and PCR reactions were prepared in a UV hood. Minimum infection rates (MIRs) were calculated by including only 1 tick per positive pool as infected. All samples positive for *Borrelia* spp. were purified using a Quantum Prep Gel

Slice Kit (Bio-Rad Laboratories, Hercules, California) and sent to SeqWright DNA Technology Services (Houston, Texas) for sequencing. Sample sequences were then compared to known sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST) accessed through the National Center for Biotechnology Information (NCBI—Bethesda, Maryland) webpage (NCBI 2006).

Statistical analysis.—Repeated-measures analysis of variance (rmANOVA) was used to test for interaction between patch size and season, and differences between patch sizes and among seasons, for the following variables: leaf-litter depth, canopy cover, ground cover, ambient temperature, relative humidity, soil moisture, soil pH, and deer activity. CWD was analyzed using 1-way ANOVA to establish statistical significance between patch sizes only (Zar 1999).

Density ($D = N/A$) of *Peromyscus leucopus* was calculated using relative abundance (individuals per 100 trap nights) as N and effective area (A) of the grid (Krebs 1999; Otis et al. 1978; Parmenter et al. 2003; Wilson and Anderson 1985). Relative abundance was chosen to represent N to incorporate trapping effort because captures in 2 of 3 large sites were consistently very low compared to small sites due to persistent trap disturbance by raccoons. One-way ANOVA was used to test for significant difference in mouse density between patch sizes. Mean densities of ticks obtained from drag sampling were expressed per m² and tested by rmANOVA for interaction and statistical significance between patch sizes and seasons.

Because relatively few nymphs were found on mice (17 of 200 juvenile ticks removed), larvae and nymphs were combined into the category of “juveniles” for analysis of tick burdens. Hereafter the term “tick burden(s)” will refer to the combination of

juvenile life stages on mice. Mean tick burdens were calculated by dividing the total number of ticks found on mice by the total number of mice captured (i.e., number of ticks per mouse). Too few mouse captures in certain seasons at some sites prevented seasonal analysis of tick burdens. Therefore, tick burdens were combined into site means to examine the effect of only patch size by 1-way ANOVA. Initially I included only ticks from newly captured mice to calculate tick burdens over the course of the study, and I noted no difference in tick burdens between patch sizes. When recaptures were used to increase sample size a similar pattern was observed. For recaptures only the 1st capture per 2- or 3-day session was included to allow reinfestation and an equal opportunity for infestation of all mice at each sampling event. Because sampling was repeated on the same experimental units, rmANOVA (Zar 1999) was used to test for significant difference in tick burdens between mouse 1) ages, 2) sexes, and 3) weights after the latter variables were categorized.

Mean infestation burdens were calculated by dividing the total number of ticks found on infested mice by the number of mice that were infested (i.e., number of ticks per infested mouse). Infestation rates (percentages of mice infested) were calculated by dividing the number of infested mice by the total number of mouse captures and dividing by 100. Mean infestation burdens and infestation rates were analyzed for treatment effects of patch size by 1-way ANOVA.

Simple linear regression (Zar 1999) was used to test for functional dependence of mean tick burden on mouse density and the relationship between tick density and mouse density. The relationship between tick burden on mice and tick density determined from drags could not be tested because of species- and life stage-specific behavior resulting in

a lack of data. Certain species and life stages of ticks were found on either drags or rodents but never both. Infection rates of *B. lonestari* could not be compared with tick burdens because the bacterium was detected only in *A. americanum*, which were found only on drags and not on mice.

Data in percent format (canopy cover, ground cover, relative humidity, soil moisture, and infestation rates) were arcsine square-root transformed before analyses. All data were tested for compliance with the assumptions of normality and homoscedasticity using Kolmogorov-Smirnov and Levene's tests, respectively. Most normality and almost all equal variance assumptions were met, but ANOVA is powerful and robust, can tolerate minor violations of assumptions (Zar 1999), and therefore was used to analyze habitat, microclimate, mouse density, tick density, tick burdens, infestation burdens, and infestation rates. All samples were random, with sites chosen based on their sizes but samples within taken randomly. Samples that could not be considered independent because data were recorded repeatedly from the same 6 sites were analyzed with rmANOVA (Zar 1999). Because of unequal sample sizes, Bonferroni multiple pairwise comparisons followed all seasonally significant ANOVA outcomes (Zar 1999). All ANOVA, regression, normality, homoscedasticity, and Bonferroni post-test analyses were performed using MINITAB Release 14 statistical software (Minitab Inc., State College, Pennsylvania). The criterion for statistical significance for all tests was $P < 0.05$.

RESULTS

Habitat and microclimate variables.—No significant interactions ($P > 0.05$) were detected between patch size and season for any habitat or microclimate variable tested by rmANOVA (Appendix I). As expected, many of these variables differed significantly ($P < 0.05$) by season (Appendix I; Figs 6-10). Leaf-litter depth ($F = 9.92$, $d.f. = 2, 12$, $P < 0.05$; Fig. 6A) and canopy cover ($F = 26.79$, $d.f. = 2, 12$, $P < 0.05$; Fig. 6B) differed among seasons, but ground cover ($F = 1.87$, $d.f. = 2, 12$, $P > 0.05$; Fig. 6C) did not. Ambient temperature ($F = 13.74$, $d.f. = 1, 8$, $P < 0.05$; Fig. 7A), relative humidity ($F = 14.35$, $d.f. = 1, 8$, $P < 0.05$; Fig. 7B), soil moisture ($F = 7.88$, $d.f. = 2, 12$, $P < 0.05$; Fig. 8A), and soil pH ($F = 12.98$, $d.f. = 2, 12$, $P < 0.05$; Fig. 8B) all differed ($P < 0.05$) among seasons. Deer usage ($F = 0.66$, $d.f. = 2, 12$, $P > 0.05$; Fig. 9) did not differ ($P > 0.05$) seasonally.

Canopy cover ($F = 15.17$, $d.f. = 1, 12$, $P < 0.05$; Fig. 6B), ground cover ($F = 56.60$, $d.f. = 1, 12$, $P < 0.05$; Fig. 6C), and soil moisture ($F = 26.73$, $d.f. = 1, 12$, $P < 0.05$; Fig. 8A) differed ($P < 0.05$) between small and large forest patches. Leaf-litter depth ($F = 4.28$, $d.f. = 1, 12$, $P > 0.05$; Fig. 6A), ambient temperature ($F = 0.02$, $d.f. = 1, 8$, $P > 0.05$; Fig. 7A), relative humidity ($F = 0.00$, $d.f. = 1, 8$, $P > 0.05$; Fig. 7B), soil pH ($F = 1.41$, $d.f. = 1, 12$, $P > 0.05$; Fig. 8B), deer usage ($F = 0.18$, $d.f. = 1, 12$, $P > 0.05$; Fig. 9),

and CWD volume ($F = 0.68$, $d.f. = 1, 4$, $P > 0.05$; Fig. 10) did not differ significantly ($P > 0.05$) by patch size.

Mammals captured and Peromyscus leucopus demography.—Seven species of small mammals were captured (Table 1). The white-footed deer mouse, *Peromyscus leucopus*, 62% of which were adults, comprised 74% of small mammals captured and 84% of total captures. Average mouse density was significantly greater in small forest patches ($F = 8.01$, $d.f. = 1, 4$, $P < 0.05$; Fig. 11).

Tick density.—Drags yielded no *D. variabilis* larvae or nymphs or *I. scapularis* larvae or adults (Table 2). The density of adult *D. variabilis* (Fig. 12A) did not differ significantly between forest patch sizes ($F = 0.00$, $d.f. = 1, 12$, $P > 0.05$) or among seasons ($F = 2.53$, $d.f. = 2, 12$, $P > 0.05$) following a lack of interaction ($F = 0.02$, $d.f. = 2, 12$, $P > 0.05$). With no interaction ($F = 0.07$, $d.f. = 2, 12$, $P > 0.05$) detected, the density of nymphal *I. scapularis* (Fig. 12B) did not differ significantly between forest patch sizes ($F = 0.11$, $d.f. = 1, 12$, $P > 0.05$) or seasons ($F = 3.53$, $d.f. = 2, 12$, $P > 0.05$). Density of adult *A. americanum* (Fig. 13A) did not differ significantly between forest patch sizes ($F = 0.71$, $d.f. = 1, 12$, $P > 0.05$) or seasons ($F = 2.11$, $d.f. = 2, 12$, $P > 0.05$) following a lack of interaction ($F = 0.50$, $d.f. = 2, 12$, $P > 0.05$). With no interaction ($F = 0.36$, $d.f. = 2, 12$, $P > 0.05$) detected, density of nymphal *A. americanum* (Fig. 13B) did not differ significantly between forest patch sizes ($F = 0.64$, $d.f. = 1, 12$, $P > 0.05$) or seasons ($F = 2.10$, $d.f. = 2, 12$, $P > 0.05$). Because of a significant interaction ($F = 4.65$, $d.f. = 2, 12$, $P < 0.05$), main effects of patch size and season on density of larval *A. americanum* (Fig. 13C) could not be analyzed. However, regression analysis revealed no functional dependence between density of mice and density of *A. americanum* larvae ($F =$

3.29, $d.f. = 1, 4$, $P > 0.05$; Fig. 14). Similarly, no functional dependence was found between density of mice and density of *A. americanum* nymphs ($F = 0.24$, $d.f. = 1, 4$, $P > 0.05$; Fig. 14) or *A. americanum* adults ($F = 0.32$, $d.f. = 1, 4$, $P > 0.05$; Fig. 14), *I. scapularis* nymphs ($F = 0.04$, $d.f. = 1, 4$, $P > 0.00$; Fig. 15), or *D. variabilis* adults ($F = 0.87$, $d.f. = 1, 4$, $P > 0.05$; Fig. 16).

Tick burdens.—All but 2 ticks (of any species) were encountered on *P. leucopus*; 2 larval *D. variabilis* were found on a single *M. musculus* (Table 1). No *A. americanum* of any life stage or adult ticks of any species were documented on mice. *I. scapularis* ($F = 3.27$, $d.f. = 1, 4$, $P > 0.05$; Fig. 17A) and *D. variabilis* ($F = 1.20$, $d.f. = 1, 4$, $P > 0.05$; Fig. 17A) burdens on newly captured mice (*P. leucopus*) did not differ between forest patch sizes. Similarly, when recaptures were included in the model, *I. scapularis* ($F = 1.23$, $d.f. = 1, 4$, $P > 0.05$; Fig. 17B) and *D. variabilis* ($F = 1.57$, $d.f. = 1, 4$, $P > 0.05$; Fig. 17B) burdens did not differ by patch size. Mean tick burdens of infested mice (Fig. 18) did not differ with patch size for either *D. variabilis* ($F = 0.00$, $d.f. = 1, 4$, $P > 0.05$) or *I. scapularis* ($F = 0.41$, $d.f. = 1, 4$, $P > 0.05$).

Infestation rates by *D. variabilis* ($F = 2.78$, $d.f. = 1, 4$, $P > 0.05$) and *I. scapularis* ($F = 2.70$, $d.f. = 1, 4$, $P > 0.05$) did not differ with patch size (Fig. 19). Neither *I. scapularis* ($F = 2.40$, $d.f. = 1, 8$, $P > 0.05$; Fig. 20A) nor *D. variabilis* ($F = 0.55$, $d.f. = 1, 8$, $P > 0.05$; Fig. 20A) burdens differed significantly by mouse (*P. leucopus*) age. No significant difference by mouse gender was detected in *I. scapularis* ($F = 0.01$, $d.f. = 1, 8$, $P > 0.05$; Fig. 20B) or *D. variabilis* ($F = 0.29$, $d.f. = 1, 8$, $P > 0.05$; Fig. 20B) burdens. *I. scapularis* ($F = 0.84$, $d.f. = 2, 12$, $P > 0.05$; Fig. 20C) and *D. variabilis* ($F = 0.61$, $d.f. = 2, 12$, $P > 0.05$; Fig. 20C) burdens did not differ significantly by mouse weight. No

functional dependence ($P > 0.05$) was found between mean juvenile *I. scapularis* ($F = 1.66$, $d.f. = 1, 4$, $P > 0.05$) or *D. variabilis* burdens ($F = 0.00$, $d.f. = 1, 4$, $P > 0.05$) and mouse density (Fig. 21).

***Borrelia* spp. minimum infection rates.**—All 188 tick individuals and pools yielded tick DNA, with further analysis confirming 9 samples as positive for *Borrelia lonestari* infection (Fig. 22). No *A. americanum* larvae, *I. scapularis*, or *D. variabilis* were infected. Specific testing revealed that all ticks were negative for *B. burgdorferi* infection for an MIR of 0% for all 3 tick species regardless of life stage (Table 3). All positive samples for *B. lonestari* originated from *A. americanum*, with an MIR of 5.9% (1 of 17) for adults and 3.4% (8 of 237) for nymphs. MIRs could not be analyzed between forest patch sizes because of inadequate tick sample sizes within each site. Because the infection rate of *B. lonestari* is so low, a minimum of 100 *A. americanum* nymphs and 10 adults per site would be required to distinguish accurate infection rates between patch sizes. With only 237 *A. americanum* nymphs and 17 adults collected among all 6 sites, inadequate sample sizes precluded confidence in the comparison of MIRs of *B. lonestari* and forest patch size.

All *Borrelia*-positive sequences aligned with known *B. lonestari* sequences in the Genbank database for further confirmation of sample results. Most but not all samples contained some unidentified nucleotides (Appendix II). However, all aligned 100% with known sequences (Appendix III). Sample sequence lengths ranging from 177-206 nucleotides aligned perfectly (100% with no mismatches or gaps) with several known sequences originated from 13 states, including Virginia, throughout the range of *A. americanum* (Appendix III).

DISCUSSION

The ecological factors influencing human risk of contracting *B. burgdorferi* and *B. lonestari* and the diseases they cause include but are not limited to 1) time of year because of tick activity (ALDF 2006), 2) habitat type and variables that influence deer movement and tick survival (Lane et al. 1991; Ostfeld 1997; Ostfeld et al. 1996a), 3) density of *P. leucopus*, which is strongly influenced by patch size and many other factors such as food availability and cover (Nupp and Swihart 1996, 1998, 2000; Ostfeld and Keesing 2000a, 2000b), and 4) presence/absence and density of host “dilution” and “rescue” species (Ostfeld and Keesing 2000a, 2000b).

Habitat characteristics may be as important as patch size in estimating Lyme disease risk. The attribute of patch size relates to densities of *P. leucopus*; competitive and predatory pressures should be reduced in small forest patches, allowing the most competent reservoir for *B. burgdorferi* to flourish (Nupp and Swihart 1996, 1998). This hypothesis is supported by Allan et al. (2002) who found greater densities of *I. scapularis* nymphs, prevalence of nymphal infection, and density of infected nymphs in smaller (<2-ha) forest patches. Greater mouse density in the small forest patches of my investigation also supports this hypothesis, but how important is reservoir density without the vectors to acquire the bacterium from that host? Characteristics of the habitat other than forest patch size determine deer activity, which is what determines

where ticks will be most dense that year and the next. A variety of habitat features also have effects on tick survival. Mast production is a major determinant of deer activity and abundance of *P. leucopus* (McShea and Schwede 1993; Ostfeld 1997, 2002; Ostfeld et al. 1996a), but many other habitat and microclimate variables share in determining tick densities and burdens once eggs are laid (Lane et al. 1991; Ostfeld 1997; Ostfeld et al. 1996a). If the environment is not conducive to tick survival, then densities and burdens will be low regardless of deer activity.

Leaf-litter depth, percent canopy cover, ambient temperature, relative humidity, soil moisture, and soil pH all differed seasonally, as expected. All of these variables play a role in the timing of tick activity. Eggs hatch and life stages begin questing when these habitat and microclimate variables permit or encourage activity (i.e., temperature is -10°C to 30°C , relative humidity $\geq 80\%$, soil moisture is near saturation, canopy $\geq 50\%$, and leaf-litter and woody vegetation are sufficient—Alder et al. 1992; Bertrand and Wilson 1996, 1997; Guerra et al. 2002; Lane et al. 1991; Lubelczyk et al. 2004; Schulze et al. 1998). However, these seasonal differences should be similar enough among sites in close proximity to assume consistent variation, as in my investigation. Tick activity began in spring when most variables were within the ranges reported above. The exceptions were relative humidity and soil moisture, but these variables were within the range to permit activity. As predicted by these variables, densities of nymphs (*A. americanum* and *I. scapularis*), which are the first life stage to become active with suitable conditions, were greatest from late May through early July in the park. Densities of *A. americanum* larvae peaked later, from late August through September, and densities of adult *A. americanum* and *D. variabilis*, although low, were

greatest in late May and early June. Too few nymphs were found attached to mice to determine time of peak activity, but larval burdens of *A. americanum* were greatest from late April through July. Predictably, these differences in activity among life stages coincided with seasonal variation in habitat and microclimate variables.

Percent ground cover did not vary seasonally, probably because once ground vegetation germinates in the spring it remains relatively unchanged throughout summer and fall. However, ground cover was greater in small patches. This is probably because of the edge effect and less canopy allowing more sunlight to penetrate to the ground in these areas in combination with greater soil moisture. The lack of seasonal variation in ground cover provided consistent refuge for ticks and mice. The lack of seasonal (and patch-size) variation in deer usage also allowed for consistency in adult and juvenile tick attachment and detachment to and from deer in patches. Therefore, any differences in tick densities or burdens that had been documented between patch sizes would not have been caused by deer.

Leaf-litter, temperature, soil pH, and relative humidity differed seasonally without differences between patch sizes, indicating that those factors varied consistently over the study area. Canopy cover and soil moisture varied with both season and patch size, suggesting a difference in habitat characteristics not measured between patch sizes, such as forest age or tree species. Greater canopy should conserve soil moisture by blocking direct sunlight, but canopy was less dense in small patches, whereas soil moisture was greatest. This may be explained by the low-lying locations and presence of creeks of 2 of the 3 small sites, even as the creeks dried up throughout the season. Large sites were more elevated and contained no visible water within study grids.

That deer usage did not differ seasonally or with patch size may help explain local trends in tick densities. The lack of seasonal variation in deer usage may lead to equal opportunity for infestation of deer by all life stages of *I. scapularis*, *A. americanum*, and *D. variabilis*. Because deer activity was similar during the 3 seasons analyzed, deer should have been similarly available for infestation by nymphs in the spring, larvae in the summer, and adults in the fall.

Seasonal variation in habitat and microclimate variables may determine tick activity and survival, but they had no apparent role in determination of tick densities in this investigation. No tick densities differed seasonally, whereas most habitat and microclimate variables did. Variables that affect deer movement, such as masting activity (Koenig and Knops 2002; McShea and Rappole 1992; McShea and Schwede 1993), have a greater effect on determining where ticks will be located and how dense they will be. If deer spend more time in one area, then ticks likely will be more dense in that location because of the greater time allowed for female ticks to drop off. In the absence or poor production of mast, vegetation and other browse become main food sources for deer. In that case, ground cover and canopy, because they influence understory growth, may have a greater effect on deer movement. However, canopy and ground cover differed with forest patch size, whereas deer usage did not, suggesting that those habitat variables did not significantly affect deer movement. Although I did not measure it, mast likely was the predominant influence among all food resources on deer movement during the year of my study (i.e., mast was regionally productive and did not differ with forest patch size).

Wilder and Meikle (2004) found greater prevalence of *I. scapularis* infestation on *P. leucopus* in large forest patches, and Allan et al. (2002) found higher densities of *I. scapularis* nymphs in smaller forest patches, neither of which is consistent with my findings. I found no difference between patch sizes in mean burdens or infestation rates of mice, leading to rejection of the hypothesis of greater *I. scapularis* burdens in smaller forest patches. Several major differences in our studies could have contributed to the different results, one of which is habitat inconsistency. Wilder and Meikle (2004) conducted their study in a heterogeneous landscape with no single dominant woody taxon, and Allan et al. (2002) worked in a maple-dominated landscape. Five of my 6 sites were oak-dominated. Another difference is how “patch” was defined by the investigators. Allan et al. (2002) considered large patches as 2-8 ha, whereas my large patches were contained within contiguous forests and bound by features like roads and streams, which are weak boundaries. In addition, Allan et al. (2002) had 14 study sites in Lyme disease-endemic New York, whereas I had 6 study sites (3 replicates each of large and small) in the low-prevalence state of Virginia. Unlike Wilder and Meikle (2004) and Allan et al. (2002), I quantified habitat and microclimate characteristics of my study sites to examine differences between small and large patches and document seasonal variations, as discussed earlier.

Wilder and Meikle (2004) did not consider the magnitude of *I. scapularis* infestation on *P. leucopus*. The authors defined “infestation” as the presence of ≥ 1 tick. I quantified tick burdens for comparison between patch sizes. Tick burdens should be quantified to demonstrate the magnitude of the infestation. An infestation rate of 80%, as defined by Wilder and Meikle, means that 80% of mice could have been infested by

only 1 tick. An infestation rate of 80% with a quantified mean tick burden of 3.2, for example, tells us that mice in that area are 3.2 times more likely to be infected with the bacterium than those with an assumed burden of 1. Higher densities of *I. scapularis* on *P. leucopus* increase the likelihood that mice will be infected with *B. burgdorferi* and a human's chance of contracting Lyme disease in that forest. In my study 16.3% of mice in large patches and 5.0% in small patches were infested with *I. scapularis*, with respective mean infestation burdens of 1.6 and 1.1 ticks/mouse. Although these differences were not significant because mouse density was greater in smaller patches, the risk of contracting Lyme disease in smaller patches is greater if *B. burgdorferi* is present. I also found 23.3% of mice in large patches and 48.0% in small patches to be infested with *D. variabilis*, with mean infestation burdens of 2.3 ticks/mouse in both cases. Although these differences were not significant, greater mouse density in smaller patches increases the risk of contracting disease transmitted by *D. variabilis* (e.g., RMSF) if the etiologic agent is present. The local prevalence of the Lyme disease spirochete could be low enough to have escaped detection in my random sample of ticks. Because no *A. americanum* were found on mice, no similar analysis could be performed for STARI.

Demographic variables of hosts are often omitted from analyses regarding tick burdens. However, Anderson et al. (2006) found that male and adult *P. leucopus* were more likely infected with *B. burgdorferi*, but infestation with *I. scapularis* was not significantly associated with infection. Apparently, spirochete infection, but not tick burdens, was greater in males and adults. Ostfeld et al. (1996b) and Davidar et al. (1989) found higher tick burdens on male *P. leucopus*. Neither *I. scapularis* nor *D.*

variabilis demonstrated selection for mouse age, gender, or weight in my investigation. Mouse density was higher in small patches, but the percentage of mice infested did not differ with patch size. I expected greater tick burdens with greater mouse density, but no functional dependence was found between mouse density and either *I. scapularis* or *D. variabilis* burdens on *P. leucopus*. Similarly, no functional dependence was found between mouse density and densities of *A. americanum* larvae, nymphs, or adults, *D. variabilis* adults, or *I. scapularis* nymphs. Deer movement and the variables that affect it may exert a greater influence on tick burdens by directly influencing tick distribution and densities, as discussed earlier. Areas with the greatest densities of ticks also should have the greatest tick burdens.

The MIRs of *A. americanum* by *B. lonestari* in this study (5.88% adult and 3.38% nymphal) are within the range (1-5% nymphal) reported by numerous researchers throughout the South (Armstrong et al. 2001; Bacon et al. 2003, 2005; Barbour et al. 1996; Clark 2004; Varela et al. 2004b). *B. lonestari* sequences from *A. americanum* matched 100% with those reported from 11 southern states – Arkansas, Florida, Georgia, Kansas, Kentucky, Maryland, Missouri, North Carolina, South Carolina, Tennessee, and Virginia – and New Jersey and New York.

My findings do not support the hypothesis of higher bacterial prevalence accompanying higher mouse densities in smaller patches. Neither greater tick burdens nor bacterial incidence coincided with high mouse density in smaller patches. However, my results support the claim that most Lyme disease cases reported in the southern United States are actually misdiagnosed cases of STARI. My laboratory results further support the distribution and incidence of *B. lonestari* in *A. americanum* throughout the

South. This, combined with the failure to isolate *B. burgdorferi* from *I. scapularis* in this study, support the claim that most Lyme-like symptoms throughout the South are caused by *B. lonestari* from *A. americanum* and not *B. burgdorferi* from *I. scapularis*. My sample size of *I. scapularis* nymphs ($n = 22$) was low compared to that of Allan et al. (2002), who examined ≥ 20 *I. scapularis* nymphs from each site for *B. burgdorferi* infection. However, I also tested larvae ($n = 49$), most of which had acquired at least a partial blood meal, and no evidence of infection was observed in this life stage. I found no similar research on *A. americanum* or STARI.

My results suggest that the risk of contracting STARI in FRSP forests is greater than that of Lyme disease, although the sample size for *A. americanum* was much greater than that for *I. scapularis*. This could have allowed for greater detection ability of *B. lonestari* compared to *B. burgdorferi*. Nevertheless, the detection of *B. lonestari* in 3.4% of *A. americanum* nymphs and 5.9% of *A. americanum* adults is evidence of the presence of the bacterium and suggests a risk, albeit low, of contracting STARI in FRSP forests.

Infection rates of ticks by *B. lonestari* may be relatively low in the South (Armstrong et al. 2001; Bacon et al. 2003, 2005; Barbour et al. 1996; Clark 2004; Varela et al. 2004b), but infection rates by *B. burgdorferi* appear even lower. Approximately 95% of Lyme disease cases occur in the northeastern and northcentral portions of the U.S., but cases are scant and scattered in the South where the vector is also established (CDC 2004; Dennis et al. 1998). In the South *A. americanum* is more abundant than *I. scapularis* (Armstrong et al. 2001; Bishopp and Trembley 1945; Childs and Paddock 2003; James et al. 2001; Varela 2004b). This is consistent with the results of my tick

sampling in which *A. americanum* comprised almost 60% of all ticks collected (drags and burdens) and *I. scapularis* only approximately 15%.

Dermacentor variabilis was negative for both *B. lonestari* and *B. burgdorferi*, leading to the rejection of the hypothesis that the species would have the ability to acquire *B. lonestari*. However, sample size was relatively low with larvae represented disproportionately. Infection with *B. burgdorferi* activates an antimicrobial defense in *D. variabilis* that rapidly kills the bacterium (Johns et al. 2000) so that even if the tick acquires the pathogen it does not maintain it. Perhaps the same is true of its infection with *B. lonestari*. That *D. variabilis* in this study did not acquire *B. lonestari* does not mean that the ability is absent. Perhaps its efficiency of acquiring *B. lonestari* is similar to that of acquiring *B. burgdorferi*. Mukolwe et al. (1992), Piesman and Happ (1997), and Piesman and Sinsky (1988) have documented low acquisition levels of *B. burgdorferi* in *D. variabilis* whereas I did not. My sample size may have been too small to detect *B. burgdorferi* or *B. lonestari* in *D. variabilis*. Further research is needed to determine the ability of *D. variabilis* to acquire *B. lonestari*, as well as its ability to maintain and transmit the pathogen.

No *I. scapularis* was infected with *B. lonestari*, causing the rejection of the hypothesis that the species would acquire the bacterium just as it acquires *B. burgdorferi*. Few tests have been conducted to determine other possible vectors of *B. lonestari* other than its principal vector, *A. americanum*. Coinfections with pathogens in different genera have been documented for *I. scapularis* (Schauber et al. 1998), so it is possible that this tick can transmit both *B. burgdorferi* and *B. lonestari*. Further testing

with larger sample sizes is necessary to determine the ability of other possible vectors to transmit *B. lonestari*.

Because *A. americanum* larvae were uninfected with *B. lonestari*, *I. scapularis* larvae also may hatch free of *B. lonestari* infection. However, no conclusion can be reached without conducting transmission trials. Because *B. lonestari* infection rates are so low (Armstrong et al. 2001; Bacon et al. 2003, 2005; Barbour et al. 1996; Clark 2004; Varela et al. 2004b), the chance is great that adult females that lay eggs are uninfected. To test for transovarial transmission ability of *B. lonestari*, larvae of adult females known to be infected must be tested.

The few potential hosts captured that were not *P. leucopus* may serve as “dilution” or “rescue” species during times of low densities of the main reservoir (Ostfeld and Keesing 2000a, 2000b). Incidental small mammal captures included the northern short-tailed shrew, eastern chipmunk, house mouse, Virginia opossum, gray squirrel, and Norway rat, all of which have demonstrated some level of reservoir competence (Craine et al. 1997; Gage et al. 1995; Gern et al. 1998; Godsey et al. 1987; Markowski et al. 1998; Mather 1993; Matuschka et al. 1997; McLean et al. 1993; Ostfeld and Keesing 2000b; Slajchert et al. 1997; Telford et al. 1990). Lizards seen in the study sites were *Eumeces fasciatus* (five-lined skink), *E. laticeps* (broadhead skink), and *Sceloporus undulatus* (northern fence lizard). Any of them may serve as “rescue” or “dilution” species (Ostfeld and Keesing 2000b), but their reservoir competencies are unknown.

MANAGEMENT RECOMMENDATIONS

The public needs information on tick-transmitted diseases and tick-borne pathogens to know what to look for and when to seek medical attention. Physicians need such information to make accurate diagnoses and prescribe proper treatment, which would be easier and faster with the development of standardized tests for both Lyme disease and STARI. Currently, we must rely on recognition of symptoms, regional location, and vector identification, which often fails because of removal or misidentification of the tick (Barbour 1996; CDC 1990a, 1990b; Oliver et al. 2000). Because the symptoms of Lyme disease and STARI are identical and other possible vectors of *B. lonestari* have not been tested extensively, we can rely only on elaborate, expensive tests for verification, which usually are not conducted (Oliver 1996). With the current database of information, misidentification of STARI for Lyme disease does not seem critical for treatment because both respond to antibiotics and STARI has even been known to dissipate untreated (Campbell et al. 1995; Felz et al. 1999; Kirkland et al. 1997; Masters et al. 1998). Nevertheless, STARI should be distinguished from Lyme disease so that both can be monitored and accurate statistics maintained.

Fortunately, the same management and prevention techniques that apply to the Lyme disease vector also apply to STARI and many other pathogens. Based on current knowledge, STARI is not nearly as focal or prevalent as Lyme disease in the United

States (Barbour 1996; Oliver 1996; Oliver et al. 2003). Even if all reported cases of Lyme disease throughout the South are actually STARI, those cases are scattered and number only in the hundreds annually compared to the thousands of Lyme disease cases focused in the northcentral and northeastern United States (CDC 2004).

To date, the infection rate of *B. lonestari* in nymphal *A. americanum* throughout the South averages about 2% (Armstrong et al. 2001; Bacon et al. 2003, 2005; Barbour et al. 1996; Clark 2004; Varela et al. 2004b) compared to an infection rate of up to 35% for *B. burgdorferi* in *I. scapularis* nymphs in focal areas (LoGiudice et al. 2003; Ostfeld 1997). Management procedures are only feasible in areas of high prevalence of a tick-borne pathogen because of the time, cost, and effort required. Fortunately, when management is called for, it affects not only the target tick species carrying the target pathogen but all tick species with all of their respective pathogens. For instance, the infection rate of Lyme disease in the northcentral and northeastern United States is high enough to justify management of its vector, *I. scapularis*.

The procedures for reducing tick abundance are not species-specific, so *I. scapularis*, *A. americanum* along the east coast, *D. variabilis* in parts of its range, and any other tick species present in the treatment area will be reduced. The prevalence of STARI may not be high enough to necessitate management for *A. americanum* throughout the southern United States, but the infection rate of another of its pathogens, like *Ehrlichia* spp., may be high in a particular region.

Host management for controlling Lyme disease or STARI may not be feasible. It has been attempted numerous times with considerable effort, various successes, and sometimes controversy (Hayes et al. 1999; Solberg et al. 2003). It is only by chance that

acaricide-treated nesting material will be used by small mammal hosts. It is perhaps more logical to target white-tailed deer because their size allows them to host literally countless ticks (all life stages), and because they are the main hosts for adult ticks, each female of which can lay up to 3,000 eggs.

In addition to meeting with public resistance, lethal elimination of deer has had various outcomes. When deer have been virtually eliminated (Wilson et al. 1988) or incrementally reduced (Deblinger et al. 1993), declines in abundance of larval and nymphal *I. dammini* (= *I. scapularis*) were documented but so were increases in abundance of adults, and the effect was often temporary. A less drastic attempt at eliminating deer is to erect fencing high enough to prevent deer from entering the entire target area (Ginsberg et al. 2002), but this would service only a relatively small area and would not exclude hosts for juvenile ticks. *P. leucopus* as well as other small mammals could come and go at will. Realistically, the 2 most important hosts of *I. scapularis* and *A. americanum*, *P. leucopus* and *O. virginianus*, are too abundant to manage directly.

My recommendation for management of Lyme disease, STARI, and any other tick-borne disease is to manage the vectors themselves, and consequently the bacteria they harbor, by way of the '4-Poster' Feeding and Application Station patented by Pound et al. (1994) of the United States Department of Agriculture (USDA—Solberg et al. 2003). This device is successful at eliminating ticks on deer, nontarget hosts, and the surrounding habitat and is environmentally friendly and publicly accepted. Each station consists of a central reservoir containing corn, which supplies a trough on either side. As deer feed at the troughs, their heads, ears, necks, and antlers (when present) contact Permethrin-treated rollers so that deer passively treat themselves with acaricide that kills

not only ticks but the disease-causing bacteria they harbor. Results so far have been remarkable. In only a 2-month trial run in 1994, a 70% decline in *A. americanum* adults on captive deer was reported in Texas (J. M. Pound and J. A. Miller, unpubl.). Pound et al. (2000) reported 92-97% control of adult and nymphal *A. americanum* on 9 fenced deer by way of a different acaricide. Most impressive is the longer-term and larger-area study by Solberg et al. (2003). Using Permethrin as the acaricide, the authors reported only 57% of sampled deer as parasitized by *I. scapularis* during the 1st year of treatment, and 0% during both the 2nd and 3rd years, compared to the control site rates of 100, 100, and 92% infestation respectively. In addition, dragging and mouse sampling documented a 91-100% reduction in all questing stages and a 70-95% decline in juvenile stages parasitizing sampled mice by the 3rd year.

Unintended but advantageous consequences of the treatment include self-application by nontarget hosts and the distribution of the acaricide into the habitat by deer and other self-treated hosts. Solberg et al. (2003) witnessed gray squirrels, raccoons, white-footed mice, eastern chipmunks, gray foxes, Canada geese (*Branta canadensis*), American crows (*Corvus brachyrhynchos*), and passerines, all of which serve as hosts for juvenile ticks and some of which are hosts also for adults, feeding at the stations. Contact with conspecifics, nests/dens, and habitat features, such as trees and brush, may disperse the acaricide further by all hosts. Permethrin is classified by the Environmental Protection Agency (EPA) as “likely to be carcinogenic to humans” (EPA 2006), and should be applied only to clothing that should be allowed to dry before wear. It is highly toxic to aquatic organisms and many insects, and adverse effects on birds and mammals have been documented, the severity of which depends upon dosage (EPA

2006). The EPA regulates application limits and mitigation procedures to keep Permethrin levels within the acceptable range (EPA 2006).

Based on the recommended treatment with a commonly used *I. scapularis* aerial pesticide for the same spatial area, Solberg et al. (2003) estimated their method to be a 157-fold decrease in the amount of pesticide introduced into the environment. The annual cost to operate 4 devices, which treated 61-74 deer, was only \$345. Its cost, noninvasiveness, and effectiveness make the '4-Poster' Feeding and Application Station a very promising option for local control of pathogen-harboring ticks.

Low disease incidence in some areas may not warrant use of the '4-Poster' device. In this case homeowners can manually remove leaf litter and woody debris from their properties (Schulze et al. 1995) and keep their lawns mowed (Falco and Fish 1988) to eliminate or reduce favorable tick habitat, refugia, and the ticks themselves. However, this requires consistent effort from landowners because of vector reinvasion and removes habitat for other favorable invertebrates.

Based on my research, I do not recommend active management for Lyme disease or STARI at FRSP. The low abundance of *I. scapularis* within the park and the absence of *B. burgdorferi* in *I. scapularis* collected suggest a remote probability of acquiring Lyme disease in FRSP. The abundance of *A. americanum* in the park is great, but the prevalence of *B. lonestari* is low. The probability of acquiring STARI is greater than the probability of acquiring Lyme disease, but prevalence is low enough to make management unjustifiable. Most visitors do not enter forested areas of FRSP, and those that do stay on trails, reducing the risk of acquiring either tick-borne bacterium.

FUTURE RESEARCH

I expected to find no adult ticks of any species on small mammals. However, it was surprising that *A. americanum* larvae and nymphs were collected from drags but never from mice, and *D. variabilis* juveniles were collected from mice but never from drags. Although perplexing, this tick behavior is not unprecedented (Cilek and Olson 2000; Stromdahl, pers. comm.). No larval or adult *I. scapularis* were collected from drags, but this simply may be due to the species' low regional abundance. Others have noted this peculiar pattern for *A. americanum* and *D. variabilis*. Cilek and Olson (2000) found only 4 immature *D. variabilis* during a 2-yr drag-sampling study, and several authors (Bishopp and Trembley 1945; Kollars et al. 2000; Sonenshine 1979) cite the lack or rarity of *A. americanum* juveniles on small mammals. Dr. Ellen Stromdahl (Tick-Borne Disease Laboratory, United States Army Center for Health Promotion and Preventative Medicine, Aberdeen Proving Ground, Maryland) states that the absence of both *D. variabilis* on drags and *A. americanum* on small mammals is not unusual (pers. comm.). However, no experiments to my knowledge have been conducted attempting to determine the reasons. Ticks are present at the site, but why are they not detected by both methods? And why do species exhibit opposite patterns of detection? Research should be aimed at the explanation of this peculiar species-specific behavior. Regardless of the causes, the species- and life stage-specific pattern of detection that emerged in this

study will allow future researchers to choose the appropriate method of collection of their target species and respective life stages.

To increase sample size for all life stages of ticks (particularly adults) for analyses of bacterial infection rates, deer should be captured so that large numbers of ticks, perhaps of several species, can be collected. My tick sample was adequate to determine accurate overall MIRs of *B. lonestari* and *B. burgdorferi* but not to compare MIRs across forest patch sizes, mouse densities, or tick densities. Sample sizes within each individual site were too low to yield the accurate site-specific mean MIRs required for statistical analyses. These MIR comparisons would have yielded vital information.

Major questions concerning reservoir competencies of STARI hosts await answers. The most efficient and abundant reservoir host for *B. burgdorferi* is *P. leucopus* (Clark et al. 2001; Donahue et al. 1987; Levine et al. 1985; Mather 1993; Ostfeld 2002; Ostfeld and Keesing 2000a, 2000b; Spielman et al. 1985; Telford et al. 1990), and the vector's terminal host is *O. virginianus*, which is reservoir-incompetent yet extremely important in the epidemiology of Lyme disease (Mather 1993; Ostfeld 1997, 2002; Ostfeld and Keesing 2000b; Telford et al. 1988). Although hosts for STARI's vector are well-documented, the reservoir competencies of those hosts are not. Is *P. leucopus* also the most efficient reservoir for *B. lonestari*? Is *O. virginianus* reservoir-incompetent for *B. lonestari* as it is for *B. burgdorferi*?

Tick burdens on nonmammalian taxa should be examined because *I. scapularis* is known to feed heavily on lizards like *Eumeces inexpectatus* (southeastern five-lined skink), *Anolis carolinensis* (green anole), *Ophisaurus ventralis* (eastern glass lizard), and *Cnemidophorus sexlineatus* (six-lined racerunner) in southern states (Levin et al. 1996;

Levine et al. 1997). *E. inexpectatus* and *A. carolinensis* have been shown to sustain *B. burgdorferi* infection in a laboratory setting (Levin et al. 1996). These species were not seen in my study sites, but *E. fasciatus*, *E. laticeps*, and *S. undulatus* were present. *S. occidentalis* (western fence lizard) is reservoir-incompetent for *B. burgdorferi* (Lane 1990). If the same is true of its eastern counterpart (*S. undulatus*), it may contribute to the “dilution effect” (Ostfeld and Keesing 2000a, 2000b) and lower Lyme disease prevalence in the region. On the other hand, if *E. fasciatus* is shown to be reservoir-competent like its western counterpart, it may contribute to the “rescue effect” (Ostfeld and Keesing 2000a, 2000b) and serve to sustain Lyme disease locally. And what are reservoir competencies of these reptiles for *B. lonestari*? What are the reservoir competencies of common avian species? Because STARI research is in its infancy, acquisition and transmission trials of other possible vectors of *B. lonestari* should continue, as should reservoir-competency tests to determine which hosts are most important in the epidemiology of STARI.

Because masting activity greatly influences Lyme disease ecology by affecting local densities of both mice and deer (Koenig and Knops 2002; McShea 2000; McShea and Schwede 1993; Ostfeld 1997, 2000; Ostfeld et al. 1996a), quantification of mast production within oak-dominated sites should be monitored. Such activity could produce important information that can be used to understand more fully the fluctuations in host numbers, parasite abundances and burdens, and infection rates.

The epidemiology of STARI is not fully understood. The recent cultivation of *B. lonestari* (Varela et al. 2004a) should allow researchers to answer many questions about the etiologic agent itself, including what distinguishes it from *B. burgdorferi*. Such

information hopefully will lead to a simple, low-cost method of diagnosis for both Lyme disease and STARI.

Major questions remain regarding STARI vectors and hosts. Because *A. americanum* was the suspected vector of *B. lonestari*, it received all of the attention in vector competency studies. It currently is unknown if *I. scapularis*, *D. variabilis*, or any other tick species is capable of acquiring, maintaining, and/or transmitting *B. lonestari*. PCR testing of other possible vectors should continue. Researchers have isolated *B. burgdorferi* from *D. variabilis*.

The ability of *B. lonestari* to be transmitted transovarially in *A. americanum* should be examined. The low infection rate (approximately 4%) of *B. lonestari* in adult *A. americanum* indicates that the chances are great that the female from which the larvae originated was uninfected. Because the infection rate is so low, transovarial transmission of *B. lonestari* in *A. americanum* is likely inefficient, as is the case for *B. burgdorferi* in *I. scapularis* (Piesman et al. 1986). However, transmission trials need to be conducted with known-infected adult *A. americanum*.

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Table 1.—Small mammals captured (all sites combined), including demographic data for *Peromyscus leucopus*. M=males, F=females.

Common name	Scientific name	Total # individuals	captures	% total captures
White-footed mouse	<i>Peromyscus leucopus</i>	90 (M=50, F=40)	62	84
Juveniles *		23 (M=14, F=9)	24	---
Subadults *		27 (M=14, F=13)	37	---
Adults *		40 (M=22, F=18)	101	---
Northern short-tailed shrew	<i>Blarina brevicauda</i>	18	18	9
Eastern chipmunk	<i>Tamias striatus</i>	8	8	4
House mouse	<i>Mus musculus</i>	2	2	1
Virginia opossum	<i>Didelphis virginiana</i>	2	2	1
Gray squirrel	<i>Sciurus carolinensis</i>	1	1	0.5
Norway rat	<i>Rattus norvegicus</i>	1	1	0.5
Totals		122	194	100

* Age class counts are based on first (new) captures. Many individuals changed age classes throughout the study.

Table 2.—Total (all patches and seasons) number of ticks counted (not all collected for analysis).

Tick Species/Life Stage	Source of ticks		Total # encountered	% of all tick encounters
	Drags	Rodents*		
<i>Amblyomma americanum</i>				
larvae	599	0	599	55.2
nymphs	238	0	238	21.9
adults	19	0	19	1.8
<i>Dermacentor variabilis</i>				
larvae	0	132*	132	12.2
nymphs	0	16	16	1.5
adults	9	0	9	0.8
<i>Ixodes scapularis</i>				
larvae	0	51	51	4.7
nymphs	20	1	21	1.9
adults	0	0	0	0
Totals	885	200	1085	100

* All rodents are *Peromyscus leucopus* except for 1 *Mus musculus* from which 2 *D. variabilis* larvae were removed.

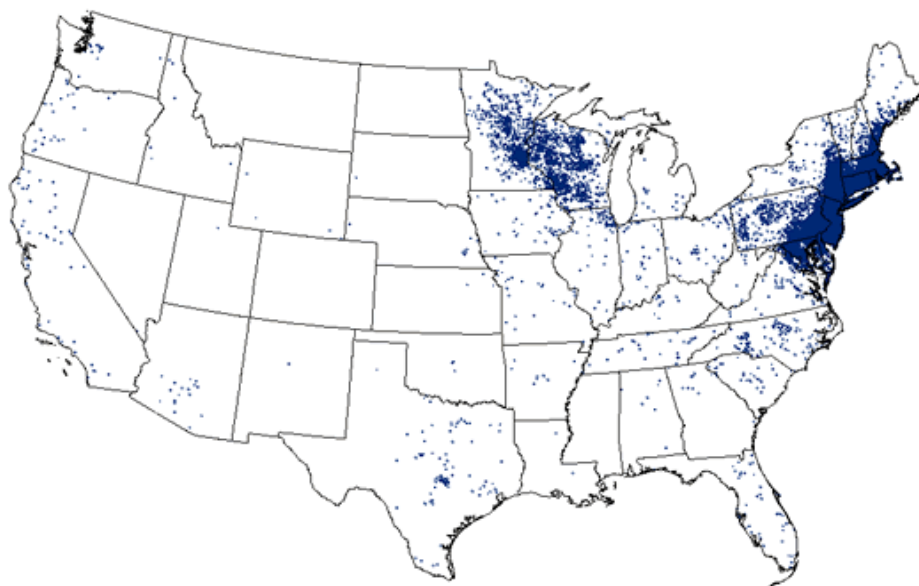
Table 3.—Total number of ticks tested for *Borrelia* spp. and minimum infection rates (MIRs).

Tick species/life stage	Source of ticks		Total	% of Sample	<i>B. burgdorferi</i>		<i>B. lonestari</i>	
	Drags	Rodents			Sample Size	MIR (%)	Sample Size	MIR (%)
<i>Amblyomma americanum</i>								
larvae	72	0	72	13.14	72	0	72	0
nymphs	237	0	237	43.25	237	0	237	3.38
adults	17	0	17	3.10	17	0	17	5.88
<i>Dermacentor variabilis</i>								
larvae	0	129*	129	23.54	129	0	129	0
nymphs	0	15	15	2.74	15	0	15	0
adults	7	0	7	1.28	7	0	7	0
<i>Ixodes scapularis</i>								
larvae	0	49	49	8.94	49	0	49	0
nymphs	20	2	22	4.01	22	0	22	0
adults	0	0	0	0	0	0	0	0
Totals	353	195	548	100	----	----	----	----

* All rodents are *Peromyscus leucopus* except for 1 *Mus musculus* from which 2 *D. variabilis* larvae were removed.

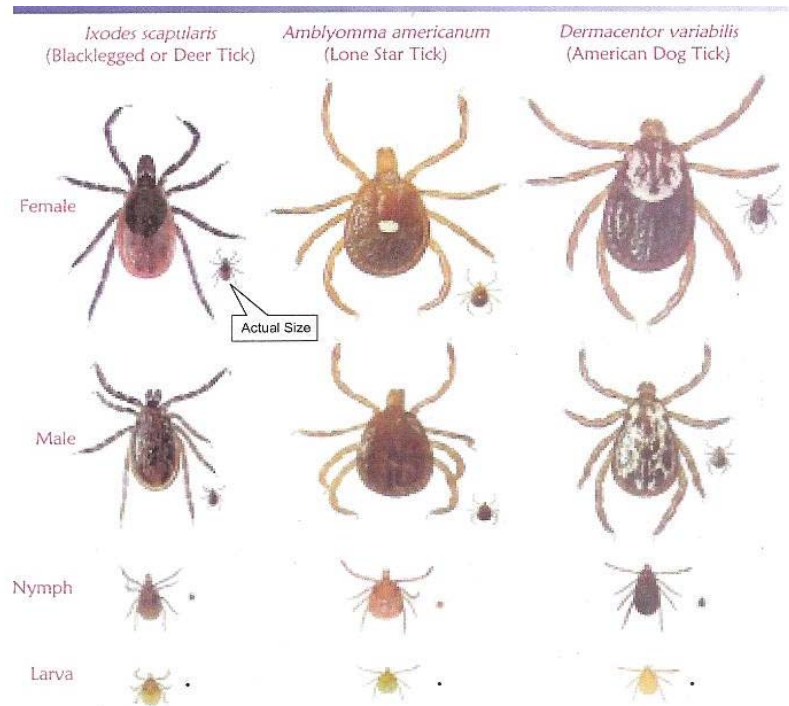
Fig. 1.—Distribution map of Lyme disease cases in the United States reported in 2004 to the Centers for Disease Control and Prevention (CDC).
http://www.cdc.gov/ncidod/dvbid/lyme/ld_Incidence.htm

Reported Cases of Lyme Disease -- United States, 2004



1 dot placed randomly within county of residence for each reported case

Fig. 2.—Illustration of adult (both female and male), nymphal, and larval stages of *Ixodes scapularis*, *Amblyomma americanum*, and *Dermacentor variabilis*. Images on the left are enlarged to show detail. Images on the right are actual size (Monmouth County Mosquito Extermination Commission, New Jersey).



Note: Larger ticks are not drawn to scale. Smaller ticks in shadow represent actual sizes.

Fig. 3.—Two-year life cycle of *I. scapularis*. The 1st year is the outer light gray area with yellow boxes and arrows, and the 2nd year is the inner dark gray area with green boxes and arrows.

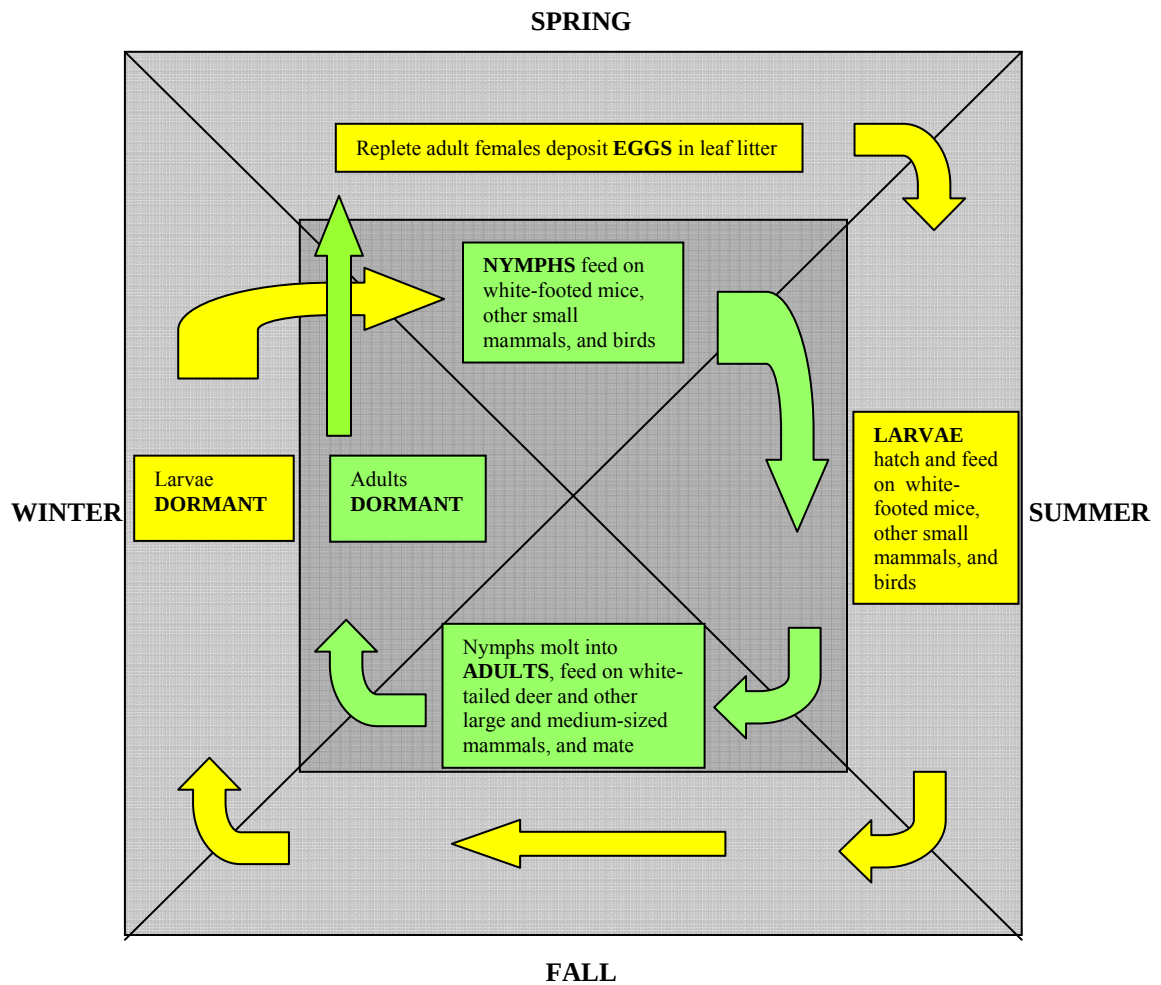


Fig. 4.—Range map of the lone star tick (*Amblyomma americanum*) in the United States (adapted from CDC; Hair and Bowman 1986).

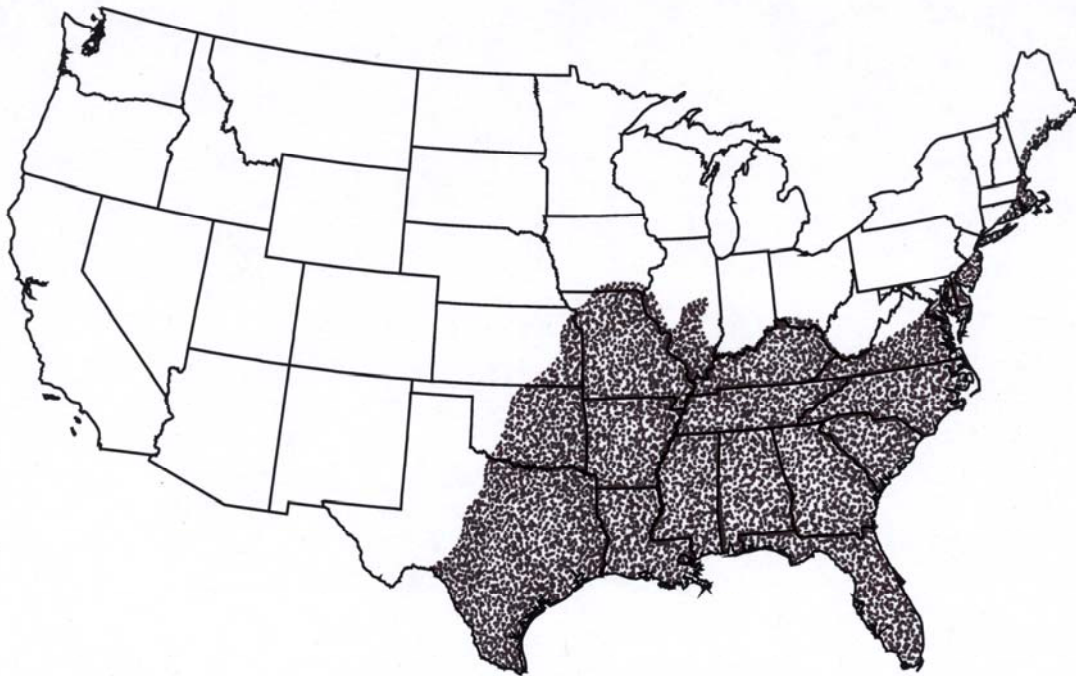


Fig. 5.—Aerial photo of study sites (yellow dots) indicating the placement of trapping grids within each of 3 small (<3-ha) and 3 large (>3-ha) forest patches. Imagery by Kucera International, Inc., Willoughby, Ohio.



Fig. 6.—Seasonal and overall A) leaf-litter depth, B) percent canopy cover, and C) percent ground cover of large (>3-ha [dark gray]) and small (<3-ha [light gray]) forest patches. $N = 3$ for all samples. Error bars represent standard errors. Seasons with different letters above the bars differed ($P < 0.05$). An asterisk (*) denotes difference ($P < 0.05$) between patch sizes.

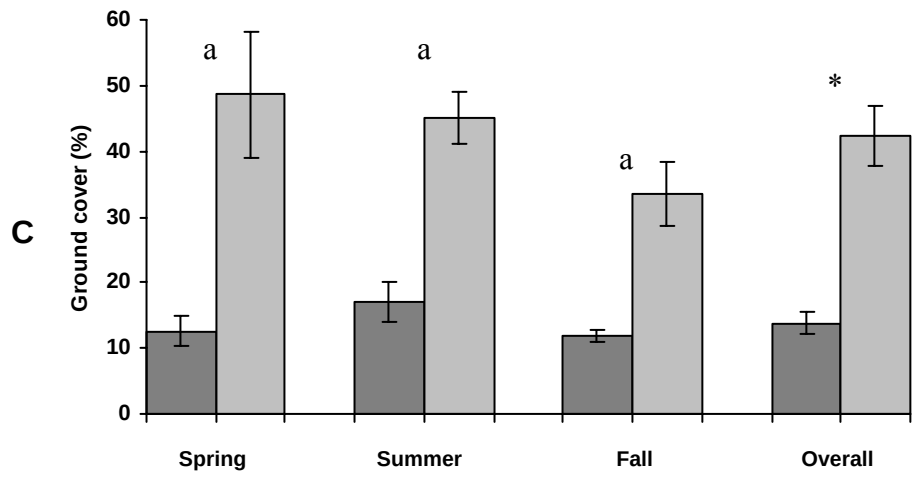
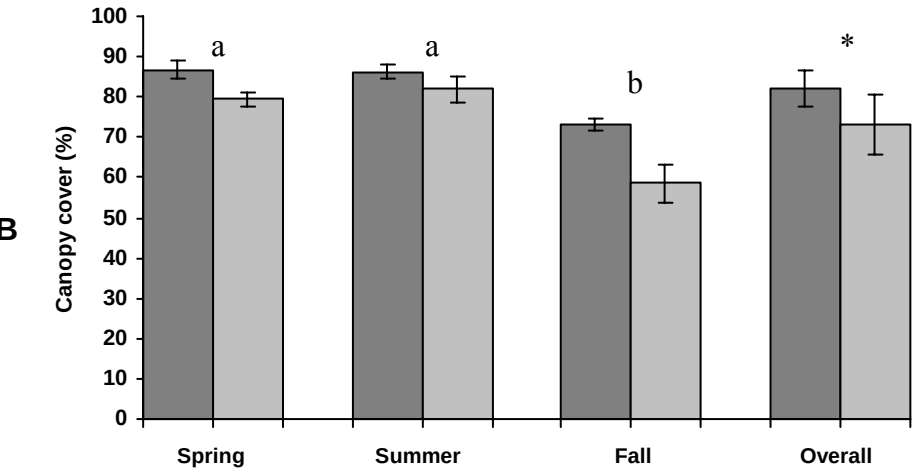
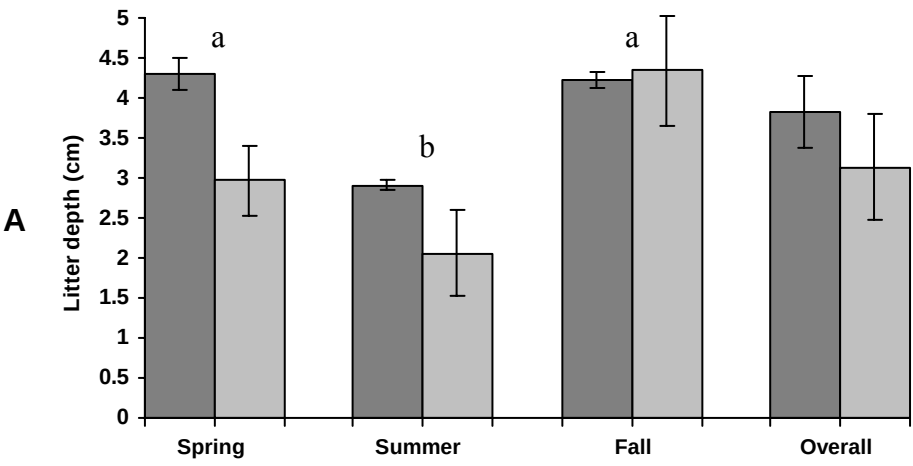


Fig. 7.—Seasonal (N = 3) and overall (N = 2) A) temperature and B) percent relative humidity of large (>3-ha [dark gray]) and small (<3-ha [light gray]) forest patches. Error bars represent standard errors. Seasons with different letters above the bars differed ($P < 0.05$).

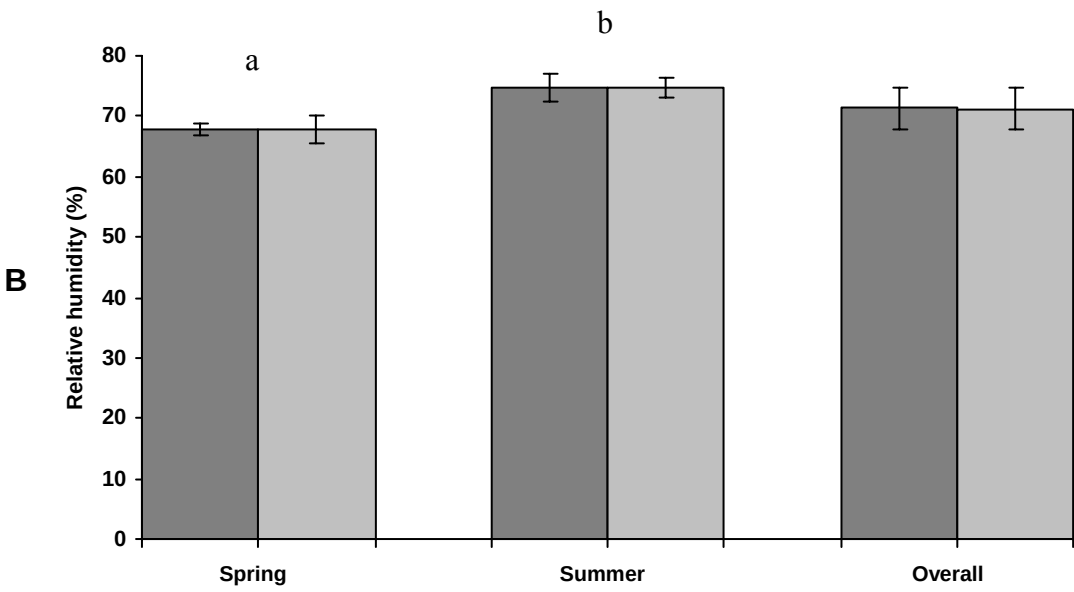
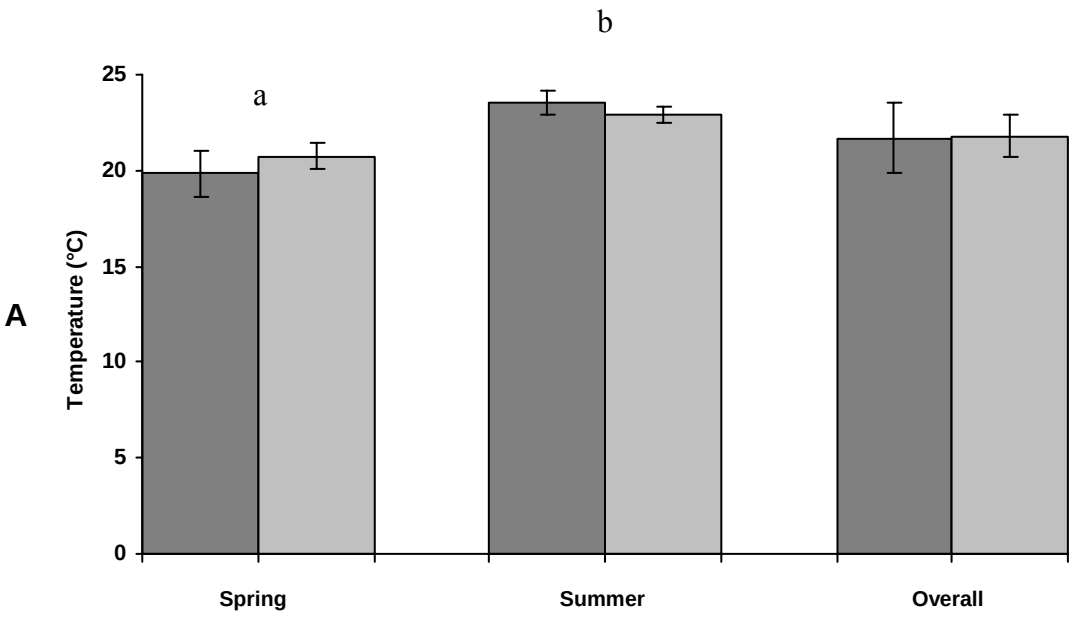


Fig. 8.—Seasonal and overall A) percent soil moisture and B) soil pH of large (>3-ha [dark gray]) and small (<3-ha [light gray]) forest patches. $N = 3$ for all samples. Error bars represent standard errors. Seasons with different letters above the bars differed ($P < 0.05$). An asterisk (*) denotes difference ($P < 0.05$) between patch sizes.

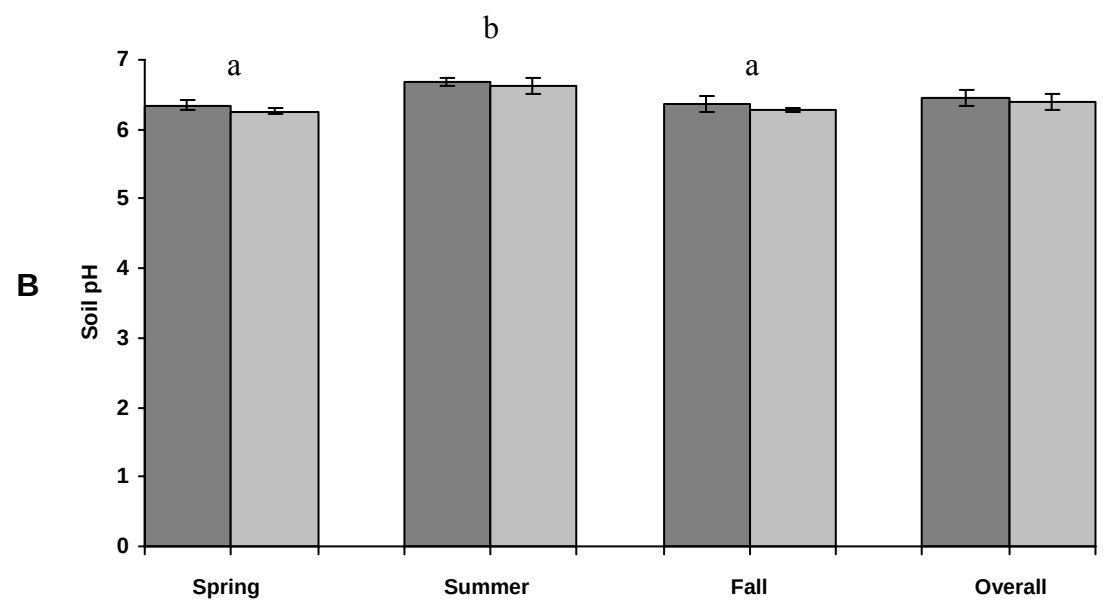
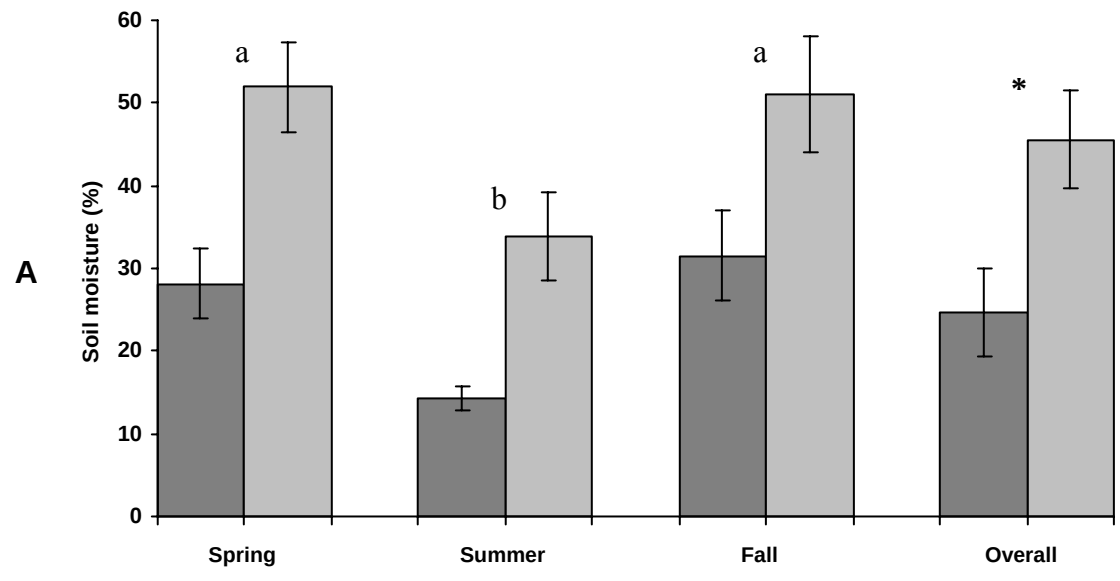


Fig. 9.—Seasonal and overall deer usage index for large (>3 -ha [dark gray]) and small (<3 -ha [light gray]) forest patches. $N = 3$ for all samples. Error bars represent standard errors.

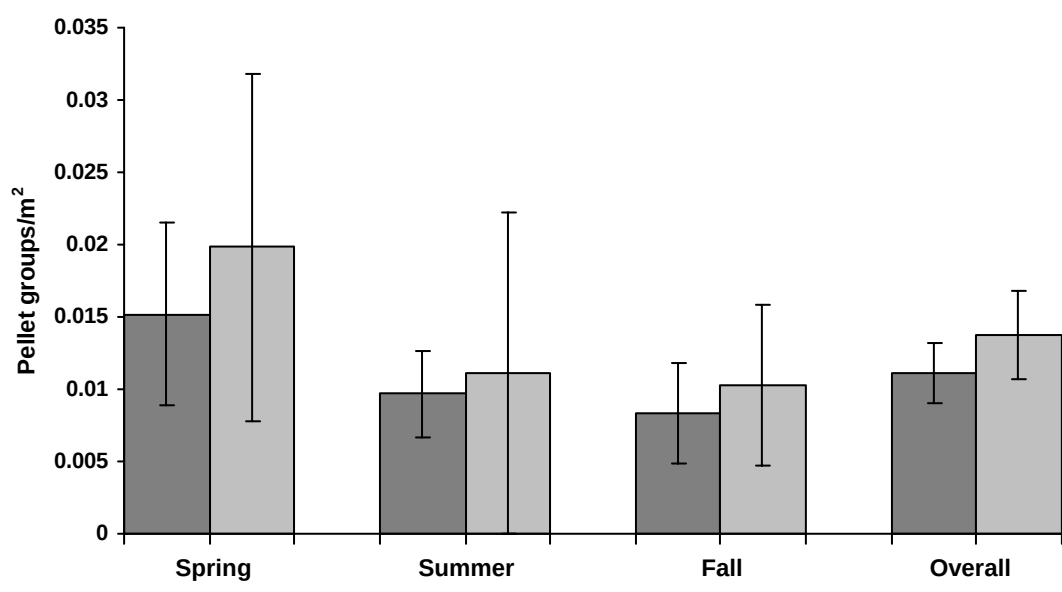


Fig. 10.—Overall coarse woody debris volume of large (>3 -ha [dark gray]) and small (<3 -ha [light gray]) forest patches. $N = 3$ for both samples. Error bars represent standard errors.

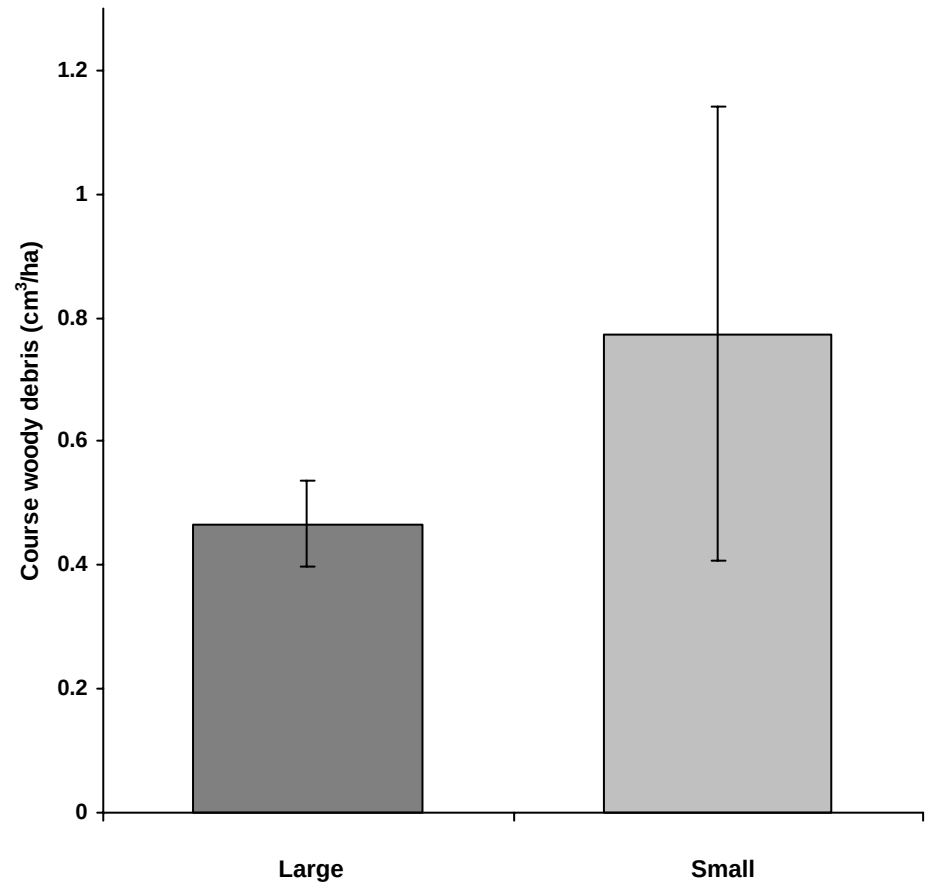


Fig. 11.—Overall mouse density of large (>3-ha [dark gray]) and small (<3-ha [light gray]) forest patches. $N = 3$ for both samples. Error bars represent standard errors. Different letters above the bars indicate a difference ($P < 0.05$) between patch sizes.

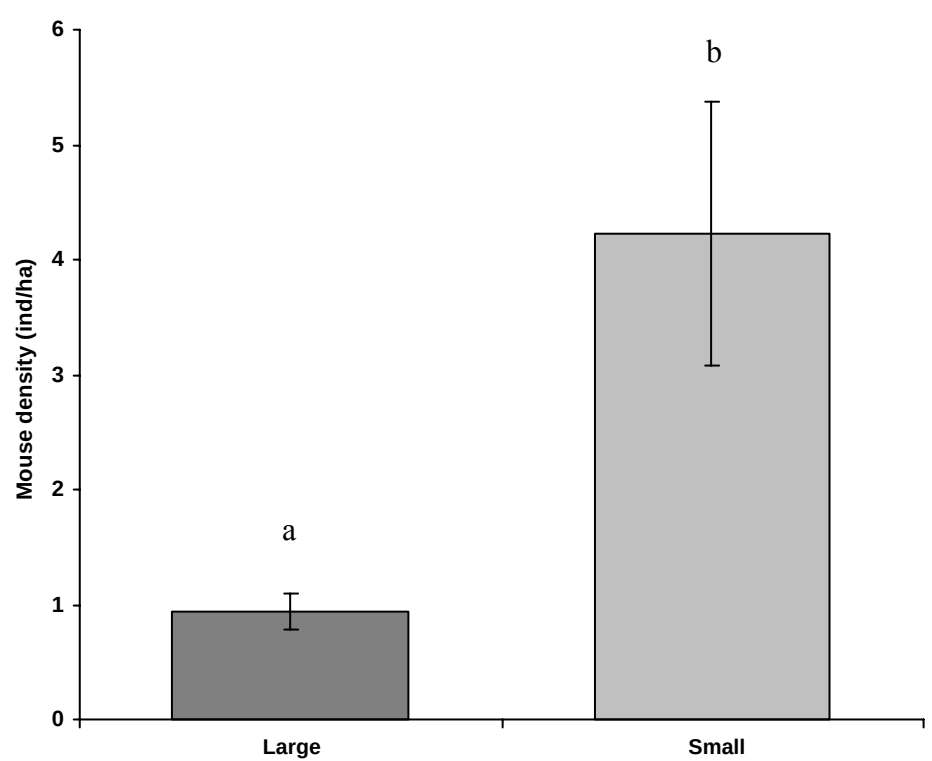


Fig. 12.—Seasonal and overall density of A) *D. variabilis* adults and B) *I. scapularis* nymphs in large (>3-ha [dark gray]) and small (<3-ha [light gray]) forest patches. N = 3 for all samples. Error bars represent standard errors.

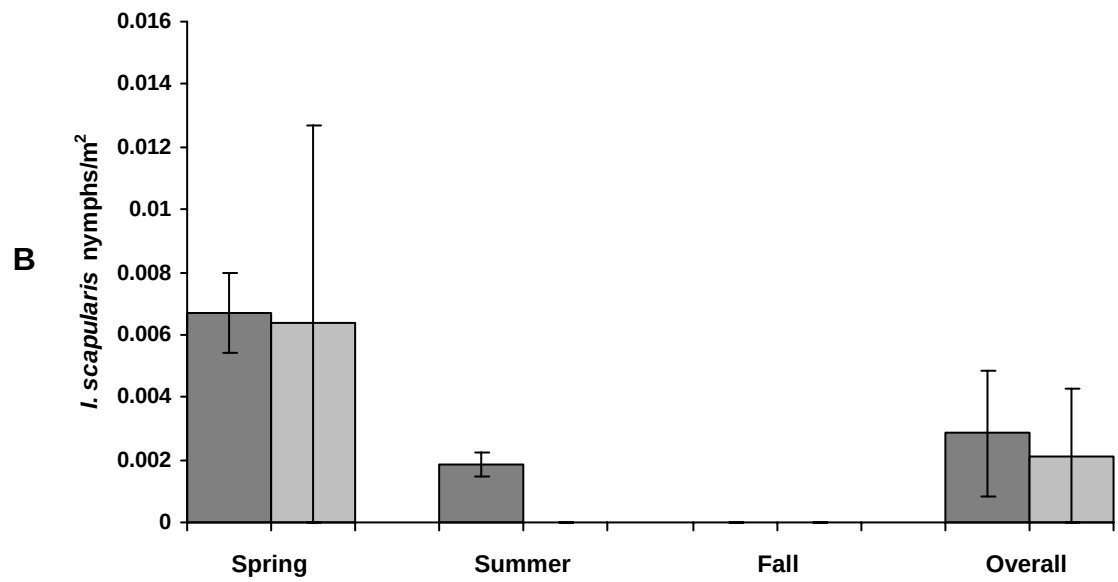
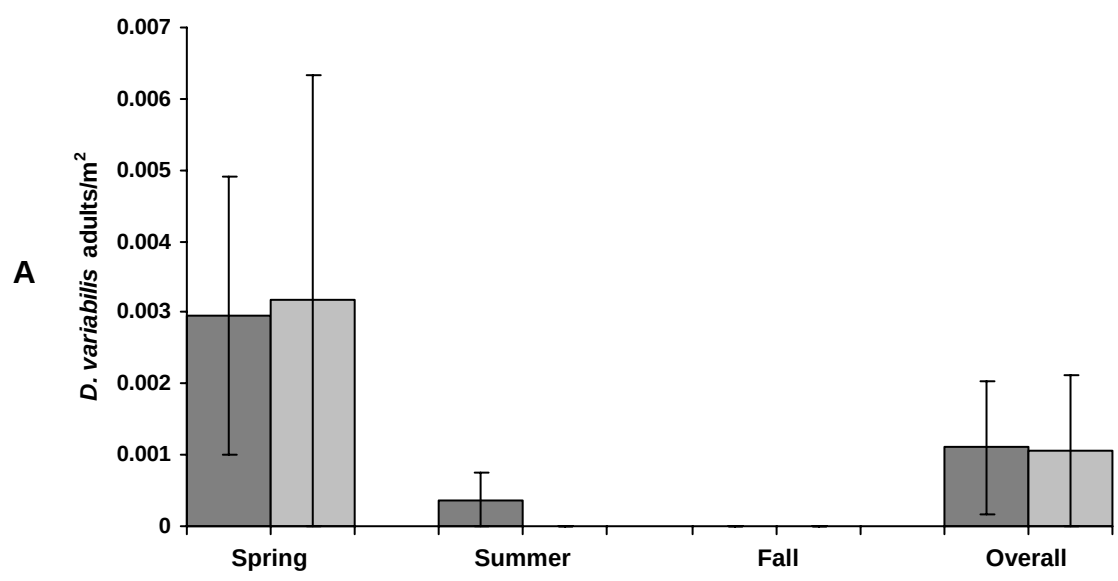


Fig. 13.—Seasonal and overall density of *A. americanum* A) adults, B) nymphs, and C) larvae in large (>3-ha [dark gray]) and small (<3-ha [light gray]) forest patches. N = 3 for all samples. Error bars represent standard errors.

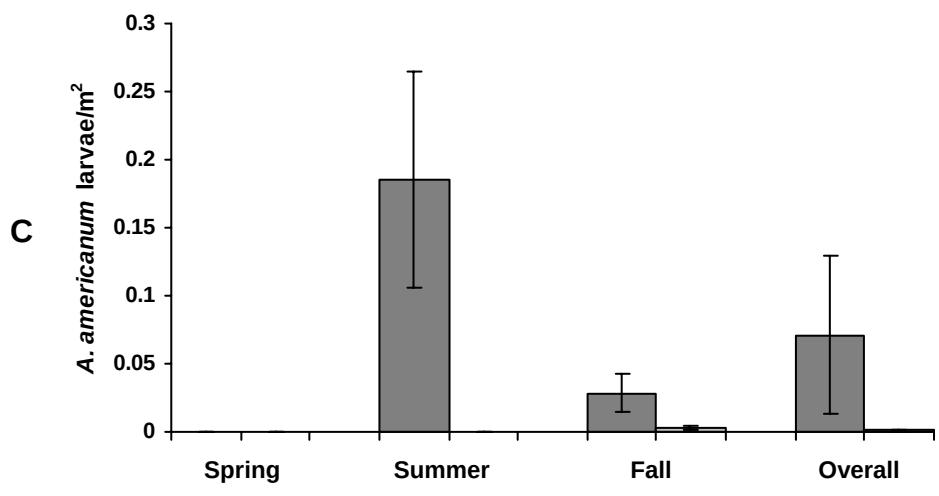
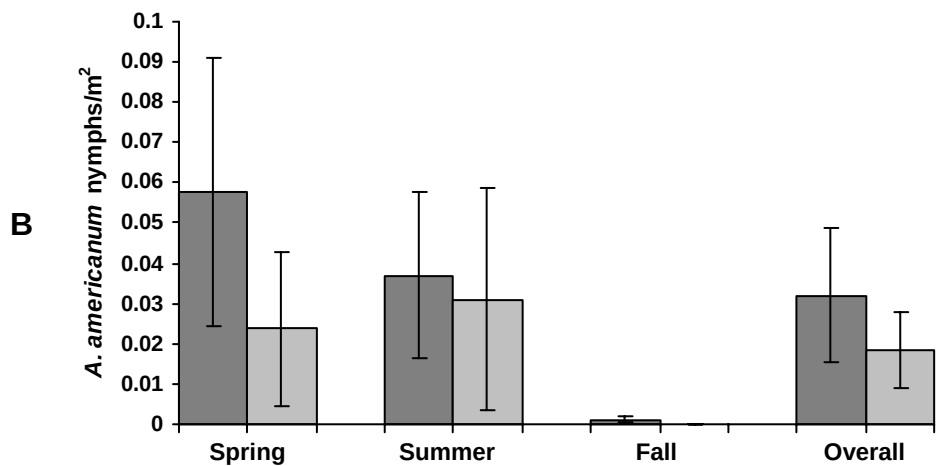
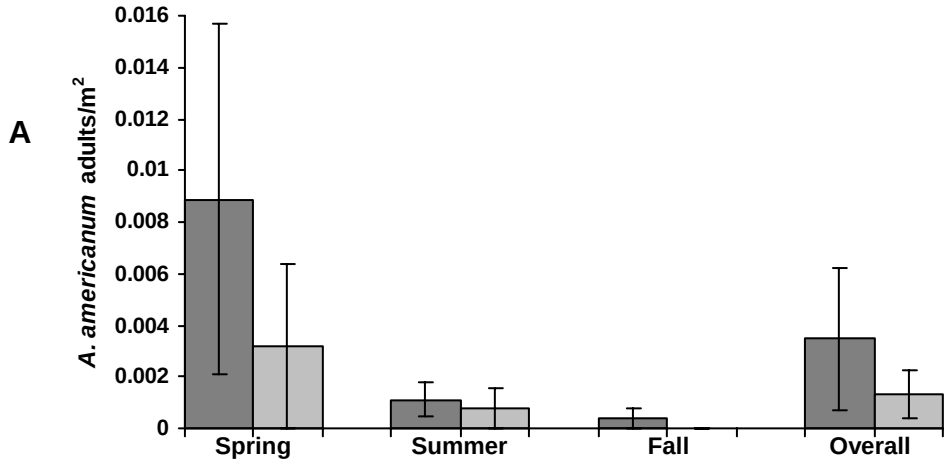


Fig. 14.—Regression of mouse density on density of *A. americanum* larvae (blue diamonds), nymphs (red squares), and adults (green triangles).

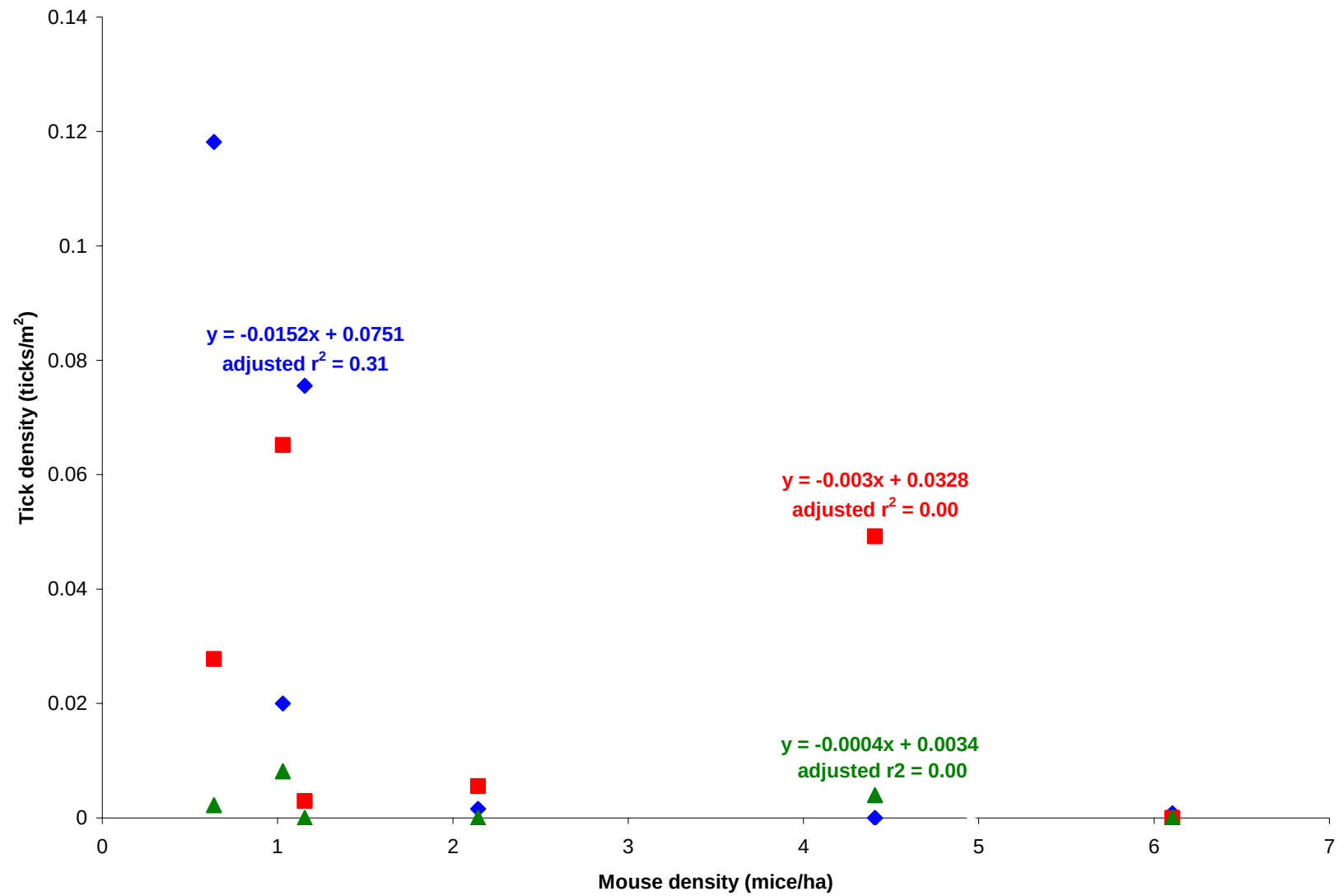


Fig. 15.—Regression analysis of mouse density on density of *I. scapularis* nymphs.

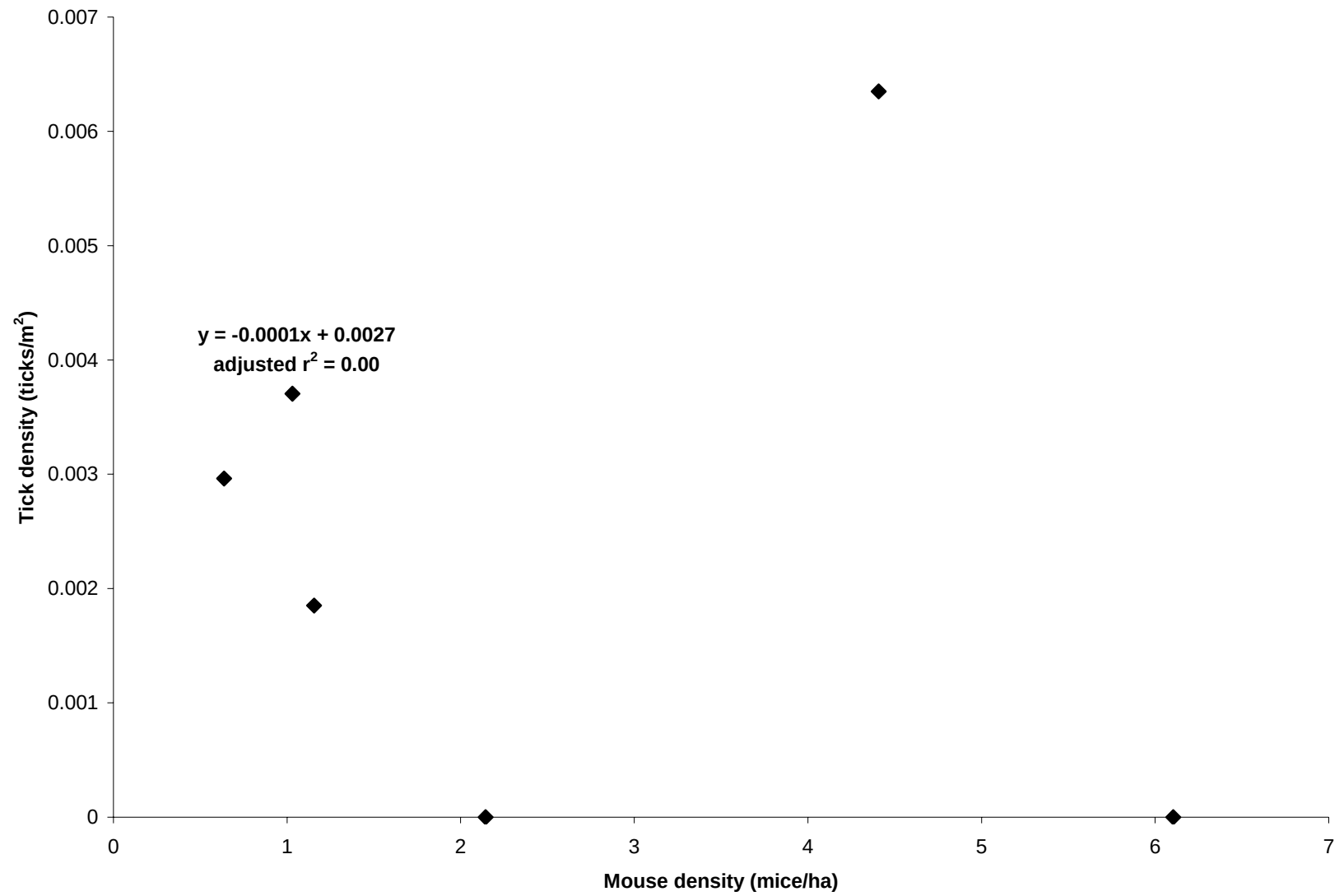


Fig. 16.—Regression of mouse density on density of *D. variabilis* adults.

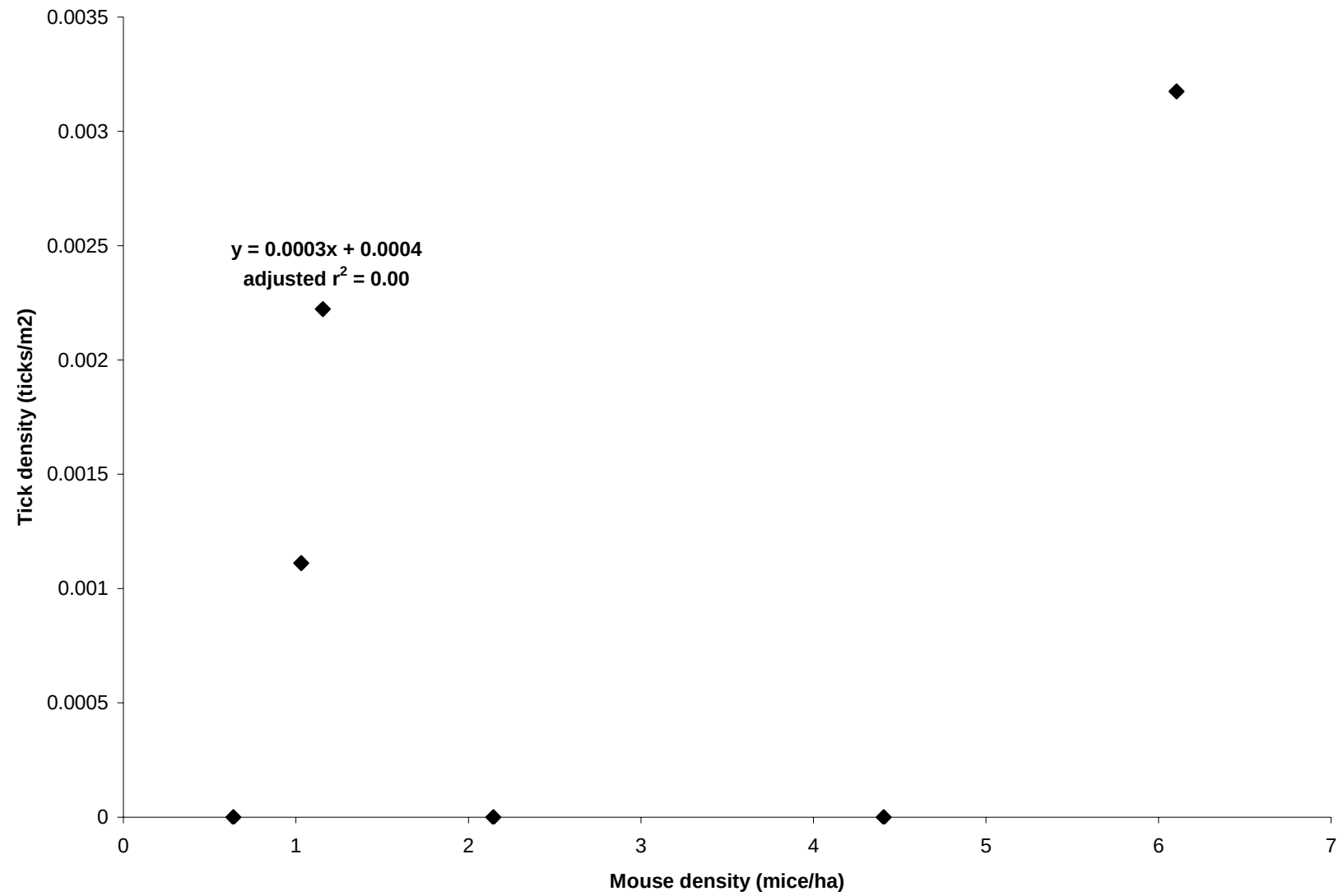


Fig. 17.—*I. scapularis* and *D. variabilis* burdens of A) newly captured mice only and B) all mice including recaptures (1st capture per trap session only) in large (>3-ha [dark gray]) and small (<3-ha [light gray]) forest patches. N = 3 for all samples. Error bars represent standard errors.

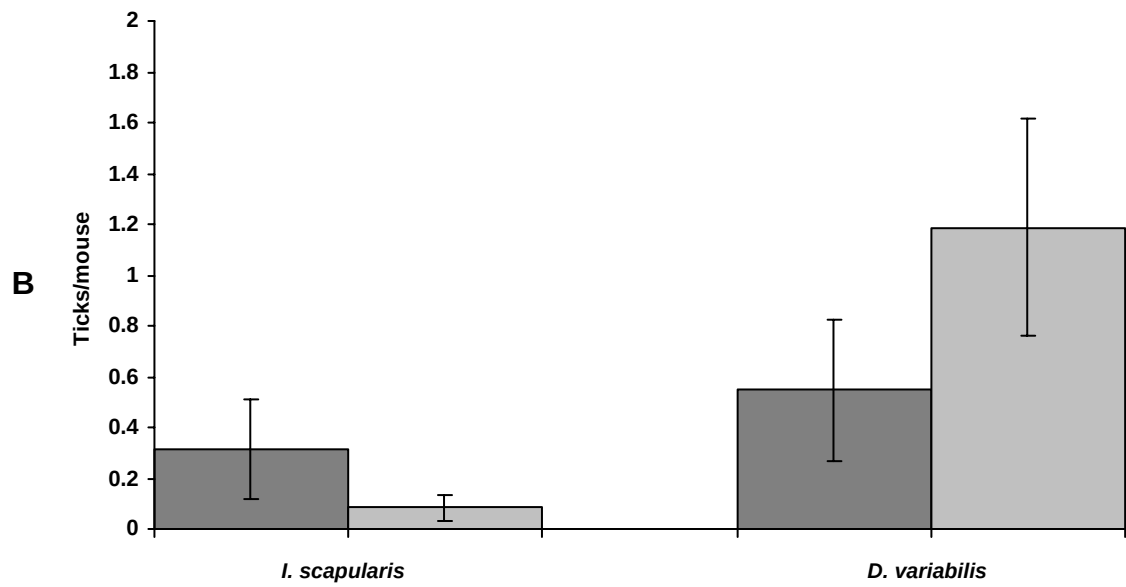
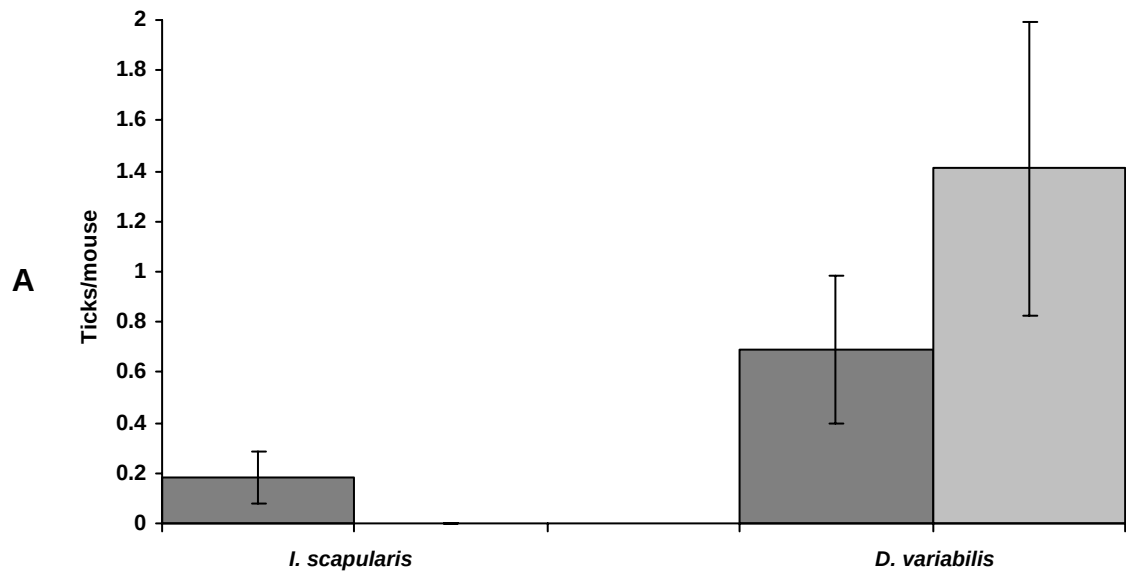


Fig. 18.—Mean burdens of *D. variabilis* and *I. scapularis* juveniles on infested mice in large (>3-ha [dark gray]) and small (<3-ha [light gray]) forest patches. N = 3 for all samples. Error bars represent standard errors.

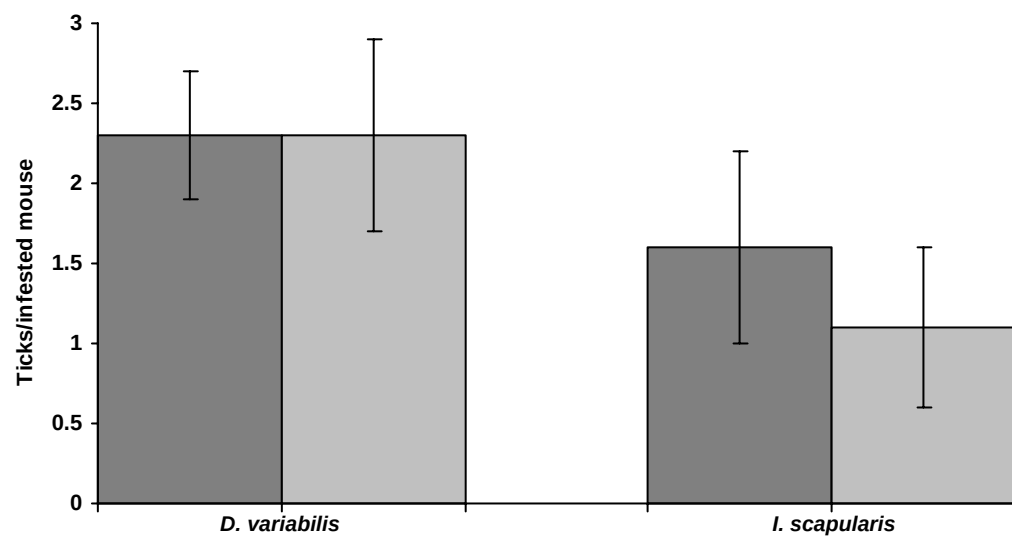


Fig. 19.—Infestation rates by *D. variabilis* and *I. scapularis* for mice in large (>3-ha [dark gray]) and small (<3 ha - light gray) forest patches. N = 3 for all samples. Error bars represent standard errors.

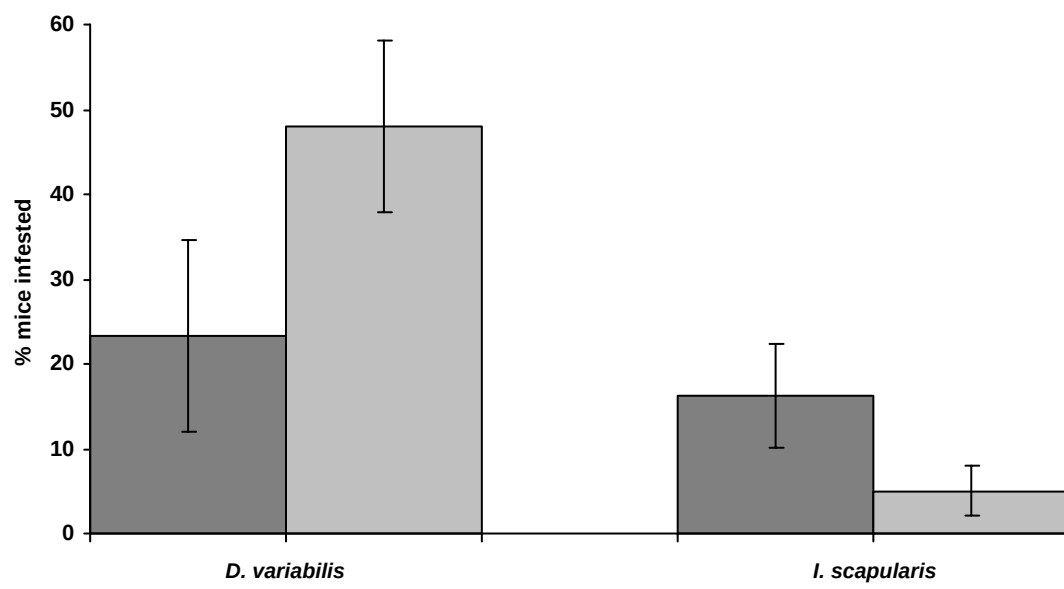


Fig. 20.—*I. scapularis* and *D. variabilis* burdens of mice, including recaptures (1st capture per session only), by mouse A) age (dark gray = juveniles and subadults combined, light gray = adults), B) gender (dark gray = male, light gray = female), and C) weight (dark gray = 0-15.0 g, light gray = 15.2-20.0 g, white $\geq$ 20.2 g). N = 3 for all samples. Error bars represent standard errors.

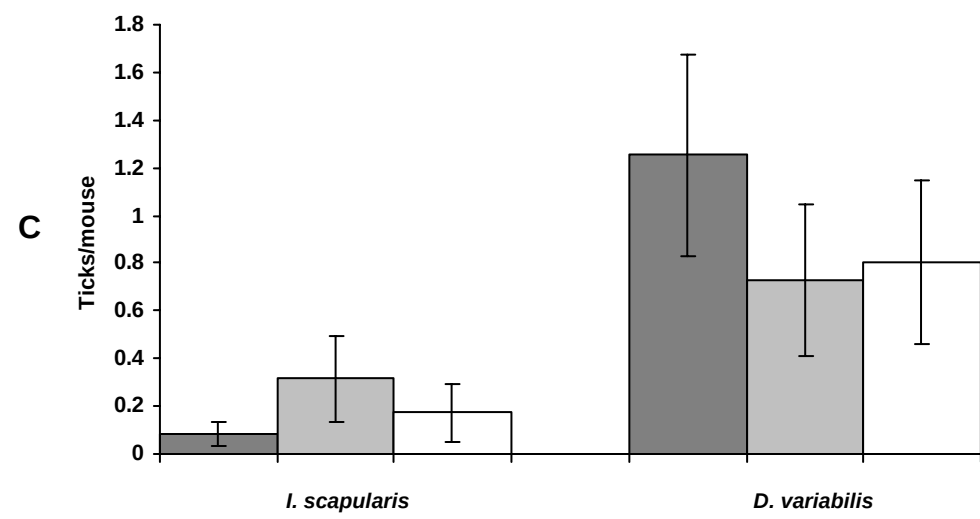
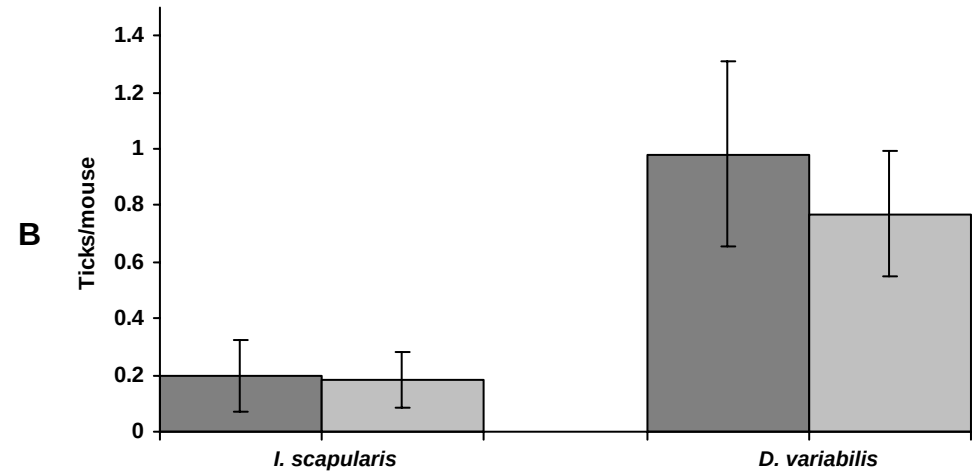
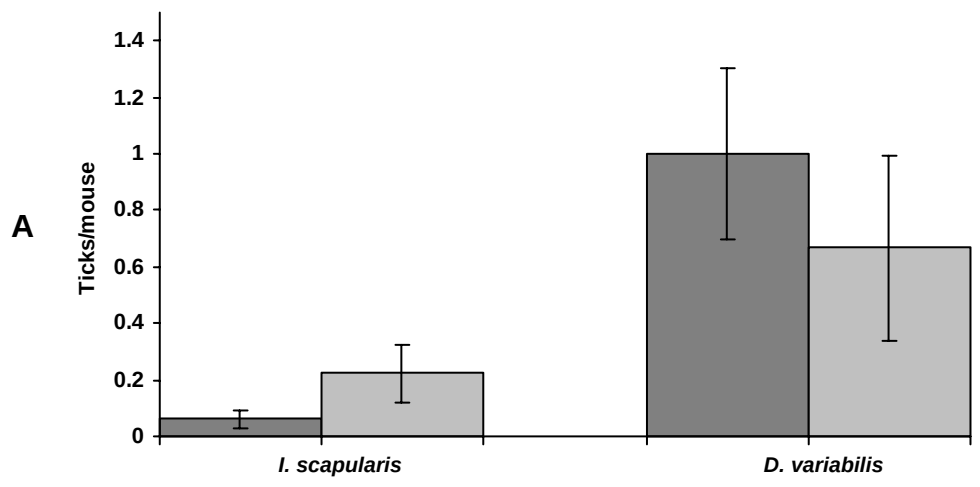


Fig. 21.—Regression of mouse density on juvenile *I. scapularis* burdens (red squares) and *D. variabilis* burdens (blue diamonds).

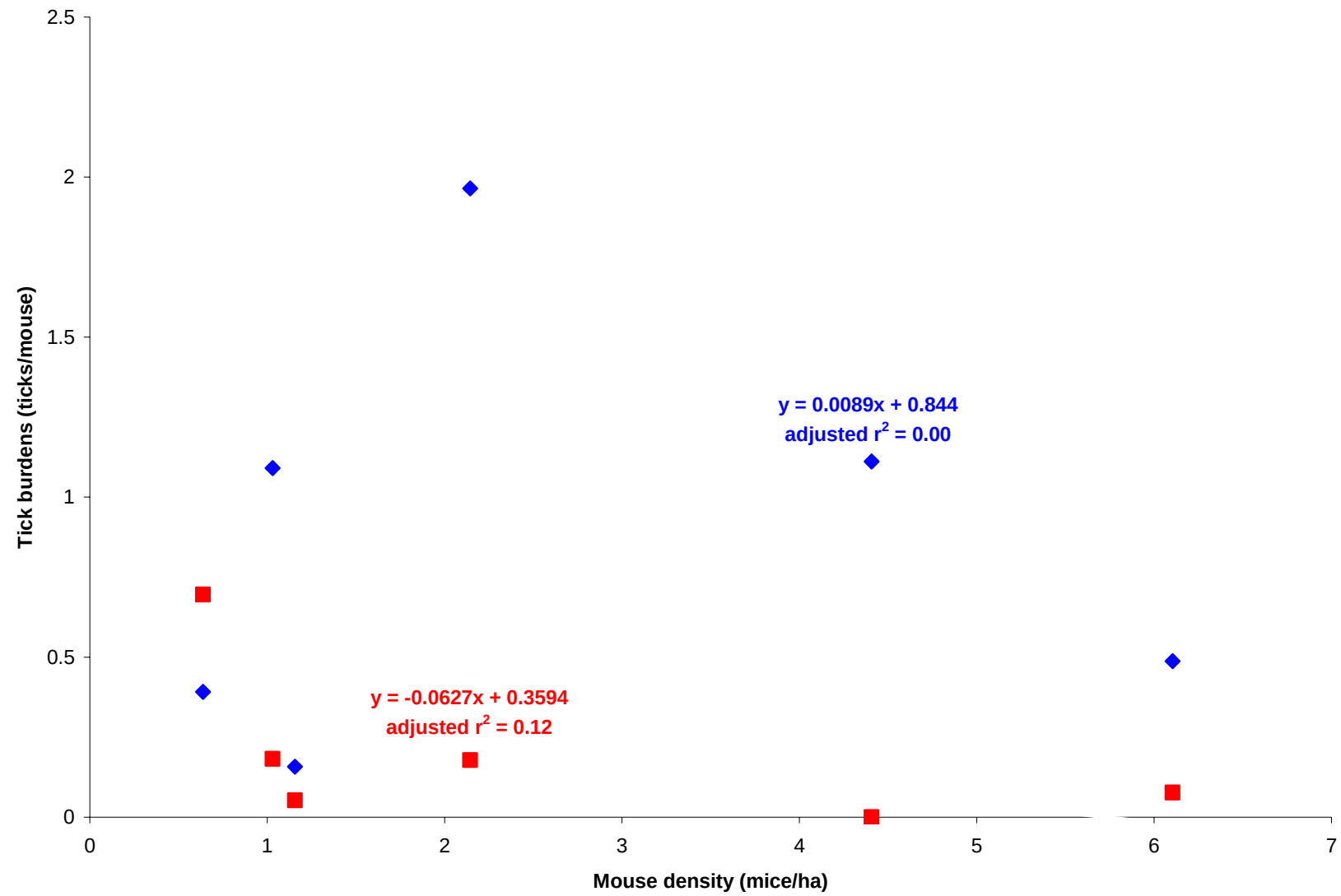
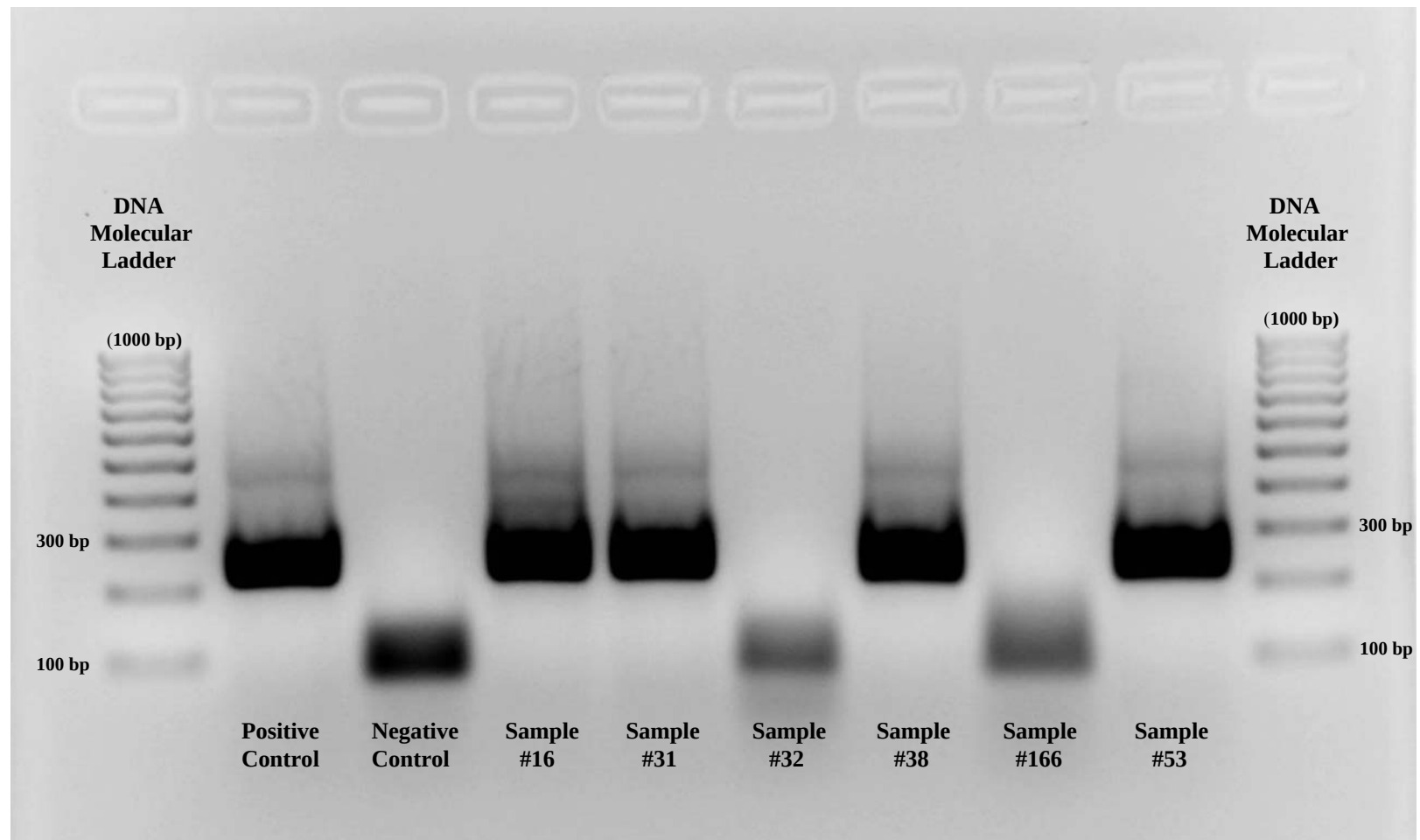


Fig. 22.—Electrophoretic results of selected DNA samples. Positive and negative controls are known *B. lonestari* and purified water, respectively. Samples #16, #31, #38, and #53 are positive with bands illuminating at approximately 240 bp. Samples #32 and #166 are negative showing only remnants of PCR reagents in the 100 bp region. Samples #58, #61, #65, #68, and #69 (not shown) also were positive (Appendix II).



Appendix I.—ANOVA results for habitat and environmental variables tested for significance ($P < 0.05$) between seasons and/or forest patch size. All ANOVAs are 2-way including tests for interactions except for the variable CWD volume, which was 1-way because season is not applicable. Any significance between forest patch sizes are between small ($N = 3$) and large ($N = 3$) patches. SP = spring, SU = summer, and FA = fall for Bonferroni post-test outcomes; seasons separated by a slash (/) were significantly different ($P < 0.05$).

Variable	Interaction			Forest Patch Size			Season			
	<i>F</i>	<i>d.f.</i>	<i>P</i>	<i>F</i>	<i>d.f.</i>	<i>P</i>	<i>F</i>	<i>d.f.</i>	<i>P</i>	Bonferroni Results
Leaf litter depth (cm)	1.66	2	0.230	4.28	1	0.061	9.92	2	0.003	SP/SU, SU/FA
Canopy cover (%)	1.08	2	0.369	15.17	1	0.002	26.79	2	0.000	SP/SU, SP/FA, SU/FA
Ground cover (%)	0.98	2	0.404	56.60	1	0.000	1.87	2	0.196	-----
CWD (cm ³ /ha)	-----	--	-----	0.68	1	0.455	-----	--	-----	-----
Deer use (pellet groups/m ²)	0.02	2	0.976	0.18	1	0.677	0.66	2	0.534	-----
Ambient temperature (°C)	0.96	1*	0.355	0.02	1	0.903	13.74	1*	0.006	SP/SU
Relative humidity (%)	0.00	1*	0.977	0.00	1	0.972	14.35	1*	0.005	SP/SU
Soil moisture (%)	0.09	2	0.910	26.73	1	0.000	7.88	2	0.007	SP/SU, SU/FA
Soil pH	0.01	2	0.995	1.41	1	0.258	12.98	2	0.001	SP/SU, SU/FA

* Equipment failure prevented the acquisition of fall data for ambient temperature and relative humidity resulting in only 1 *d.f.* for those variables regarding season and interaction.

Appendix II.—Nucleotide sequences of the nine samples positive for *Borrelia lonestari*. N indicates an undetermined nucleotide.

Sample #	# Base Pairs	Sequence
16	760	NNNATATANCCCAGCATCACTAACTGGAGCACAAGCTTCATGGACATTGAGAGTTCAGGTA GGTGCAAATCAGGATGAAGCAATTGCTGTTAATATTTTCTCAACTAATGTTGCAAATCTTTT TGGTGGAGAAGGTGTTCAAGCGGCTCCAGCTCAAGAAGAGGGTGCACAACAGGAAGGAGT TCAACCAGCTCCAGCTCAAGGTGGGATTAGCTCAANTGATGTTTCTGACCNNTGTGAACAG CTCCNNCTCCTCCACCCAAATCTACCAGNCTGTGTCTCGTATTGTCTGCGGNCATCCCGAGG NAGGGGGGCTGAANATCAAGTCTCTCANCTGNNATGANGACNACANCTACNAAGCCCTCT TTGNAGGCNATGGCACTGANGANNATGCTGAAACCTTCTNTGACANCTCTNCNNCTCCTTA CTGCAATGATTTNATGAANAANTTGNANTCTAGNCCTCTTTCCCNCAATTANCTGNAAAGCT CTGANGCCNCTGCTCGTTGGNAANATCCTGTATACNCCTNACNCTCCAGCCNCNANGNNNG TCATGGCTGANGTGAACAANACCTTCCNNNNACTGGCTGTGTTCCNTGATCNNNNANNGCNT GTGNNAANNNCTCNNCCCCNNTATCTGGACCTTCATGNNNAACNNCCAAGANATGNACCTT GTCCGGATGCNNNNNNNACNNNNNGNACCNTNANCNCTTTTNNAACANNNNNTTGNNTNNN TTANATTGNNCNNCNGNAATCNNNNNNN
31	516	NNATATACCCAGCATCACTAACTGGAGCACAAGCTTCATGGACATTGAGAGTTCAGGTAGG TGCAAATCAGGATGAAGCAATTGCTGTTAATATTTTCTCAACTAATGTTGCAAATCTTTTGT GTGGAGAAGGTGTTCAAGCGGCTCCAGCTCAAGAAGAGGGTGCACAACAGGAAGGAGTTC AACCAGCTCCAGCTCAAGGTGGGATTAGCTCAACTNNCCNNNCCTTCCCNACCNTATCTCG CTCNTGCANTGTGTCANCTGNNCAANCTANAACCCNTANCTACNGANGTCTGGCTCATC AACNANNCCNTANAAGTCTGNATGANATGAAGTTCTGGNCTGNNATTGNGTTCCTGNAN TTACTIONGANCNTTTNANCTNCCCCATCNTGTCATGTACAANATCCNCATGTACNTTNAC NANGTGNANANNACNAATNAAATCANNNATGGNTCCTGNNACCCTNNTCCNCNANCTATC CCCTNTNAGNACNTGCNNNACTCCNGNN

Appendix II.—Continued.

Sample #	# Base Pairs	Sequence
38	433	NATATACCCAGCATCACTAACTGGAGCACAAGCTTCATGGACATTGAGAGTTCAGGTAGGT GCAAATCAGGATGAAGCAATTGCTGTTAATATTTTCTCAACTAATGTTGCAAATCTTTTTGG TGGAGAAGGTGTTCAAGCGGCTCCAGCTCAAGAGGGTGCACAACAGGAAGGAGTTC AACC AGCTCCAGCTCAAGGTGGGATTAGCTCAANNNCTNNCCTGCTANTGNTCANCCTNNANCNN NNAAACCTGCTGCCCTACNNTGATCCCNACNTGCCGTTTCNCCTCCTGTCCGGGTCTNCTNN NNNNACNNTCCTGCACTGCTTCCTNATTANCTCNCNCCTCNCCTNANCCNCCCTGGCANCA CCCTGNGCNNCNNTCNTCTANTTNNCNCNTCNCCTGCCCTACCTCCTGTGNCNTTCATNNNN NGNC
53	204	NNNATATACCCAGCATCACTAACTGGAGCACAAGCTTCATGGACATTGAGTGCAAATCAG GATGAAGCAATTGCTGTTAATATTTTCTCAACTAATGTTGCAAATCTTTTTGGTGGAGAAGG TGTTCAAGCGGCTCCAGCTCAAGAAGAGGGTGCACAACAGGAAGGAGTTC AACCAGCTCC AGCTCAAGGTGGGATTAGCTCA
58	358	ANNNATATACCCAGCATCACTAACTGGAGCACAAGCTTCATGGACATTGAGAGTTCAGGTA GGTGCAAATCAGGATGAAGCAATTGCTGTTAATATTTTCTCAACTAATGTTGCAAATCTTTT TGGTGGAGAAGGTGTTCAAGCGGCTCCAGCTCAAGAGGGTGCACAACAGGAAGGAGTTC A ACCAGCTCCAGCTCAAGGTGGGATTAGCTCAANNNGCNCNNNCNTTNNNGNCCCTNNNNA NNNTNNNNNTNNTGNATNNNNNNNTTNNCCCNCCGNANATCTAGAANCCANCGNNNCCACN TNAATNNTTNNCGTTCCCAACCACNNTNNNNNNNCANCCTNNNNNNNTTNNCATNNN
61	214	NATATACCCAGCATCACTAACTGGAGCACAAGCTTCATGGACATTGAGAGTTCAGGTAGGT GCAAATCAGGATGAAGCAATTGCTGTTAATATTTTCTCAACTAATGTTGCAAATCTTTTTGG TGGAGAAGGTGTTCAAGCGGCTCCAGCTCAAGAAGAGGGTGCACAACAGGAAGGAGTTC A ACCAGCTCCAGCTCAAGGTGGGATTAGCTCA

Appendix II.—Continued.

Sample #	# Base Pairs	Sequence
65	215	CTATATACCCAGCATCACTAACTGGAGCACAAGCTTCATGGACATTGAGAGTTCAGGTAGG TGCAAATCAGGATGAAGCAATTGCTGTTAATATTTTCTCAACTAATGTTGCAAATCTTTTG GTGGAGAAGGTGTTCAAGCGGCTCCAGCTCAAGAAGAGGGTGCACAACAGGAAGGAGTTC AACCAGCTCCAGCTCAAGGTGGGATTAGCTCA
68	212	ATATACCCAGCATCACTAACTGGAGCACAAGCTTCATGGACATTGAGAGTTCAGGTAGGTG CAAATCAGGATGAAGCAATTGCTGTTAATATTTTCTCAACTAATGTTGCAAATCTTTTGGT GGAGAAGGTGTTCAAGCGGCTCCAGCTCAAGAAGAGGGTGCACAACAGGAAGGAGTTCAA CCAGCTCCAGCTCAAGGTGGGATTAGCTC
69	215	NATATACCCAGCATCACTAACTGGAGCACAAGCTTCATGGACATTGAGAGTTCAGGTAGGT GCAAATCAGGATGAAGCAATTGCTGTTAATATTTTCTCAACTAATGTTGCAAATCTTTTGG TGGAGAAGGTGTTCAAGCGGCTCCAGCTCAAGAGGGTGCACAACAGGAAGGAGTTCAACC AGCTCCAGCTCAAGGTGGGATTAGCTCANN

Appendix III.—Sequences in the Genbank database aligning 100% with *Borrelia*-positive sample sequences.

Sample #	Accession #	% Identity	Sequence Length	Alignment Length	Alignment Location	# Mismatches	# Gaps	Source Location
16	AY850063	100	990	206	481-686	0	0	MO
16	AY654941	100	299	206	15-220	0	0	FL
16	AY552536	100	269	206	37-242	0	0	GA
16	AY552533	100	321	206	30-235	0	0	GA
16	DQ485687	100	465	206	1-206	0	0	NY
16	DQ480218	100	479	206	15-220	0	0	NY
16	AY237721	100	315	206	40-245	0	0	MD
16	AY237720	100	315	206	40-245	0	0	MD
16	AY237719	100	315	206	40-245	0	0	MD
16	AY237718	100	315	206	40-245	0	0	VA
16	AY237717	100	315	206	40-245	0	0	AR
16	AY237716	100	315	206	40-245	0	0	MD
16	AY237715	100	315	206	40-245	0	0	VA
16	AY237714	100	315	206	40-245	0	0	VA
16	AY237712	100	315	206	40-245	0	0	VA
16	AY237707	100	333	206	44-249	0	0	NJ
16	AY237706	100	315	206	40-245	0	0	KS
16	AY237705	100	315	206	40-245	0	0	NC
16	AY237703	100	315	206	40-245	0	0	KY
16	AY237702	100	315	206	40-245	0	0	KY
16	AY237701	100	315	206	40-245	0	0	VA
16	AY237699	100	315	206	40-245	0	0	VA
16	AY237696	100	315	206	40-245	0	0	NJ
16	AY237695	100	315	206	40-245	0	0	KY
16	AY237693	100	315	206	40-245	0	0	VA
16	AY237692	100	315	206	40-245	0	0	VA

Appendix III.—Continued.

Sample #	Accession #	% Identity	Sequence Length	Alignment Length	Alignment Location	# Mismatches	# Gaps	Source Location
16	AY237691	100	315	206	40-245	0	0	VA
16	AY237690	100	315	206	40-245	0	0	NJ
16	AY237689	100	315	206	40-245	0	0	NJ
16	AY237688	100	315	206	40-245	0	0	VA
16	AY237683	100	315	206	40-245	0	0	VA
16	AY237681	100	315	206	40-245	0	0	VA
16	AY237679	100	333	206	44-249	0	0	VA
16	AY237678	100	333	206	44-249	0	0	VA
16	AY237677	100	333	206	44-249	0	0	VA
16	AY237674	100	315	206	40-245	0	0	VA
16	AY237672	100	315	206	40-245	0	0	VA
16	AY237671	100	333	206	44-249	0	0	VA
16	AY237670	100	315	206	40-245	0	0	VA
16	AY237668	100	315	206	40-245	0	0	VA
16	AY237667	100	333	206	44-249	0	0	VA
16	AY237666	100	315	206	40-245	0	0	VA
16	AY237665	100	315	206	40-245	0	0	VA
16	AY237664	100	315	206	40-245	0	0	VA
16	AY237663	100	315	206	40-245	0	0	VA
16	AY237657	100	315	206	40-245	0	0	VA
16	AY237656	100	333	206	44-249	0	0	VA
16	AF408410	100	423	206	15-220	0	0	TN
16	AF273671	100	333	206	43-248	0	0	NC or MD
16	AF273670	100	333	206	43-248	0	0	NC or MD
16	AF538848	100	257	187	1-187	0	0	AR
16	AF538847	100	257	187	1-187	0	0	AR

Appendix III.—Continued.

Sample #	Accession #	% Identity	Sequence Length	Alignment Length	Alignment Location	# Mismatches	# Gaps	Source Location
16	AF538852	100	257	187	1-187	0	0	GA
16	AF538850	100	257	187	1-187	0	0	NC
16	AY362359	100	436	177	40-216	0	0	VA
31	DQ485687	100	465	206	1-206	0	0	NY
31	AF538848	100	257	187	1-187	0	0	AR
31	AF538847	100	257	187	1-187	0	0	AR
31	AF538852	100	257	187	1-187	0	0	GA
31	AF538850	100	257	187	1-187	0	0	NC
31	AY362359	100	436	177	1-177	0	0	VA
38	DQ473306	100	457	199	457-259	0	0	NY
38	AY552535	100	254	184	1-184	0	0	GA
38	AF538851	100	254	184	1-184	0	0	NC
38	AF538849	100	254	184	1-184	0	0	NC
38	AF538846	100	254	184	1-184	0	0	SC
53	DQ485687	100	465	206	1-206	0	0	NY
53	AF538848	100	257	187	1-187	0	0	AR
53	AF538847	100	257	187	1-187	0	0	AR
53	AF538852	100	257	187	1-187	0	0	GA
53	AF538850	100	257	187	1-187	0	0	NC
53	AY362359	100	436	177	1-177	0	0	VA
58	DQ473306	100	457	199	457-259	0	0	NY
58	AY552535	100	254	184	1-184	0	0	GA
58	AF538851	100	254	184	1-184	0	0	NC
58	AF538849	100	254	184	1-184	0	0	NC
58	AF538846	100	254	184	1-184	0	0	SC
61	DQ485687	100	465	206	1-206	0	0	NY

Appendix III.—Continued.

Sample #	Accession #	% Identity	Sequence Length	Alignment Length	Alignment Location	# Mismatches	# Gaps	Source Location
61	AF538848	100	257	187	1-187	0	0	AR
61	AF538847	100	257	187	1-187	0	0	AR
61	AF538852	100	257	187	1-187	0	0	GA
61	AF538850	100	257	187	1-187	0	0	NC
61	AY362359	100	436	177	1-177	0	0	VA
65	DQ485687	100	465	206	1-206	0	0	NY
65	AF538848	100	257	187	1-187	0	0	AR
65	AF538847	100	257	187	1-187	0	0	AR
65	AF538852	100	257	187	1-187	0	0	GA
65	AF538850	100	257	187	1-187	0	0	NC
65	AY362359	100	436	177	1-177	0	0	VA
68	DQ485687	100	465	206	1-206	0	0	NY
68	AF538848	100	257	187	1-187	0	0	AR
68	AF538847	100	257	187	1-187	0	0	AR
68	AF538852	100	257	187	1-187	0	0	GA
68	AF538850	100	257	187	1-187	0	0	NC
68	AY362359	100	436	177	1-177	0	0	VA
69	DQ473306	100	457	199	457-259	0	0	NY
69	AY552535	100	254	184	1-184	0	0	GA
69	AF538851	100	254	184	1-184	0	0	NC
69	AF538849	100	254	184	1-184	0	0	NC
69	AF538846	100	254	184	1-184	0	0	SC