THE EFFECTS OF LONG-TERM PRESCRIBED BURNING ON TICKS AND TICK-BORNE PATHOGEN PREVALENCE

by

ELIZABETH RAIMEY GLEIM

(Under the Direction of Michael J. Yabsley)

ABSTRACT

Identifying methods for control of tick populations and reducing risk of tick-borne disease is critical. One promising method is prescribed fire. To determine the impacts of long-term prescribed burning on tick and pathogen dynamics, 21 sites in southwestern Georgia with varying burn regimes (burned surrounded by burned [BB], burned surrounded by unburned [BUB], unburned surrounded by burned [UBB], and unburned surrounded by unburned [UBUB]) were sampled monthly for 2 years. Simultaneously, data on other variables known to affect tick abundance (e.g. host abundance, vegetation structure, and micro- and macroclimate) were collected. In total, 47,184 ticks were collected, of which, 99% were *Amblyomma americanum* followed by *Ixodes scapularis* (0.7%) and fewer numbers of *A. maculatum, Dermacentor variabilis,* and 1 *I. brunneus.* Long-term prescribed burning significantly reduced tick abundance regardless of other variables and burn regimes. Furthermore, tick species composition differed with *A. americanum* dominating in UBUB sites and *A. maculatum* dominating in BB sites. To determine whether *Solenopsis invicta* (red imported fire ants [RIFA]) (typically associated with burned areas) and/or habitat type were driving tick dynamics in burned

areas, an experimental field study in which *A. americanum* and *A. maculatum* nymphs were placed in enclosures assigned to three treatments (burned, RIFA present; burned, RIFA absent; and unburned, RIFA absent) was performed. *A. maculatum* survival was significantly greater than *A. americanum* survival in burned habitats (regardless of RIFA) and *A. americanum* survival was significantly greater in unburned habitat as compared to burned habitat. To further investigate impacts of burning on tick dynamics, all ticks collected during the field study were tested for pathogens including *Rickettsia* spp., *Borrelia* spp., *Anaplasma phagocytophilum*, and *Ehrlichia* spp. Prevalences of each organism (*Rickettsia* 6.7-66.7%, *Borrelia* 0-2.2%, *E. chaffeensis* 0-1.1%, *E. ewingii* 0-7.2%, and Panola Mountain *Ehrlichia* 0-3.8%) were similar to other studies in the Southeast. While long-term prescribed burning did not alter pathogen prevalence, the reduction in tick counts resulted in the density of infected ticks being 35 times lower in burned sites as compared to UBUB sites. Thus, long-term prescribed burning is an effective tool for controlling tick populations and ultimately reducing risk of tick-borne disease.

INDEX WORDS: ticks, *Amblyomma*, *Rickettsia*, *Ehrlichia*, *Borrelia*, *Anaplasma*, prescribed burning, prevalence, *Solenopsis invicta*

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DEDICATION

I would like to dedicate this to the many friends, family, and mentors in my life who have supported me and helped me to be where am I today. Thank you to for your love and support and for instilling in me the drive to continue learning and pursuing my passions.

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CHAPTER 1

INTRODUCTION

The overall goal of this dissertation was to determine the effects of long-term prescribed burning on ticks and tick-borne pathogen prevalence and the potential implications that this may have for human and domestic animal health. Prescribed fire is a common forest management tool, particularly in the Southeast. As the impacts of human land modification are being implicated in the spread and emergence of diseases around the world (Allan et al. 2003, Patz et al. 2005, Goodin et al. 2006), it is critical that we understand the impact that land management practices have on wildlife, domestic animal and human disease prevalence.

Tick-borne diseases are a significant public and veterinary health concern. For example, tick-borne bacteria that cause Lyme disease, ehrlichiosis and Rocky Mountain spotted fever in humans results in over 20,000 cases/year. Important tick-borne diseases of domestic animals include babesiosis, ehrlichiosis, and hepatozoonosis. Due to the dramatic increase in incidence of tick-borne disease and tick population distributions, it has become increasingly important to identify ways to reduce risk of tick-borne diseases. Specifically, identifying financially viable methods which could be applied easily on a landscape extent and result in reductions of entire tick populations would be highly desirable and effective in reducing risk of tick-borne diseases. Perhaps one of the most promising methodologies fitting these criteria is prescribed burning.

It has been shown that fire initially decreases the number of ticks present at a burned site (Hoch et al. 1972, Wilson 1986, Stafford et al. 1998); however, the degree to which a tick population is decreased is correlated to burn intensity and amount of ground litter removed by fire (Stafford et al. 1998). A more controversial question is whether fire ultimately causes a long-term decrease in tick abundance and furthermore, how regular prescribed burns may affect tick populations. Previous studies have suggested that burn intensity, host prevalence, microclimate and ground litter all likely play an important role in tick abundance and re-colonization of an area after a burn (Duffy et al 1994, Ostfeld et al. 1995, Schulze et al. 1995, Stafford et al. 1998, Perret et all 2004). Generally, most studies on the effects of fire on ticks have found that ticks are less abundant in burned versus unburned sites (Jacobson and Hurst 1979, Drew et al. 1985, Wilson 1986, Cully 1999). However, a number of studies have reported that ticks often re-colonize an area within approximately 1 year of burning (Cully 1999, Stafford et al. 1998). In contrast, other studies have observed increased numbers of ticks or tick-borne pathogens at burn sites, leading to speculation that the increase in host prevalence due to and in conjunction with ground cover regeneration after a fire results in increased numbers of ticks (Mather et al. 1993, Cilek and Olson 2000).

These studies show that the dynamics of tick abundance are complex. While many studies have addressed the effects of prescribed burns on tick and/or tick-borne pathogen prevalence, it is suspected that the conflicting results reported in these various studies are likely due to failure to account for many of the factors known to affect tick population dynamics. For example, microclimate, vegetation and host abundance are known to affect tick presence and abundance in an area, yet no study on prescribed burns

has been conducted while accounting for all of these factors (Ostfeld et al. 1995, Schulze et al. 1998, Schulze et al. 2002, Perret 2004).

Furthermore, past studies often failed to simulate operational prescribed fire management regimes, which typically involve burning hundreds to thousands of acres every 1-5 years on a regular basis. Instead, studies were often performed at sites that had not been burned, which is not typical of most wildlife management areas managed by prescribed burns (Hoch et al. 1972, Drew et al. 1985, Mather et al. 1993, Bailey and Whitham 2002). Furthermore, burns often times were not performed over large areas or for more than one burn cycle (Jacobson and Hurst 1979, Mather et al. 1993, Davidson et al. 1994, Cully 1999).

In this study, I evaluated tick dynamics at sites on which prescribed fire had been utilized for 10 years or more. Through accounting for vegetation structure, microclimate, burn interval, and relative abundance of hosts, I evaluated the potential role that fire plays in the species diversity and abundance of ticks present at each site and the implications that this may have for public health. I hypothesize that there will be positive correlations with ground litter abundance, host prevalence, and tick prevalence. Finally, I hypothesize that tick-borne pathogen prevalence will not be affected by burning due to availability of reservoir hosts in burned and unburned habitats. However, even if prevalence of pathogens is not affected by burning, burning will likely cause a significant decrease in the abundance of infected ticks in an area, which ultimately will lead to a decreased risk to human and domestic animal health. To test these hypotheses, I completed these four specific objectives:

1. Determine the impacts of various long-term prescribed burn regimes on tick abundance and species composition while accounting for host abundance, vegetation structure and microclimate.
2. Determine the pathogen prevalence of ticks in burned and unburned habitat.
3. Determine whether factors unique to burned habitat, including habitat type and presence of *Solenopsis invicta* (red imported fire ant, also known as, RIFA) affect survival of *A. americanum* and *A. maculatum*.
4. Conduct an epidemiologic study to identify the species of ticks infesting people in Georgia, prevalence of pathogens in these ticks, and risk factors associated with human tick bites.

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CHAPTER 2

LITERATURE REVIEW

Prescribed Burns: An Integral Tool for Land Management

Controlled burns have become a well-accepted form of ecosystem management and wildfire prevention (Haines et al. 2001, Kirkman et al. 2001, Fernandes and Botehlo 2003). Fires reduce fuel load and cause regeneration and diversification of the understory of forests (Towne and Owensby 1984, Rego et al. 1991, Sparks et al. 1998, Kirkman et al. 2001, Knapp et al. 2005). Furthermore, when utilized over long periods of time, fire can reduce or eliminate the mid-story, resulting in a dense and diverse understory and semi-open canopy which is ideal for pine ecosystems (Rego et al. 1991, Brockway and Lewis 1997). The immediate regeneration of understory after a burn and the long-term density and diversity of the understory provides habitat and resources for many animal species (Hobbs and Stowart 1984, Sparks et al. 1998, Kirkman et al. 2001). In the Southeast, the value of prescribed burns is particularly apparent where forests are dominated by fire-adapted and –dependent tree species, such as longleaf pines (*Pinus palustris*). Thus, the Southeast has the largest number of prescribed burns in the country (Virginia Department of Forestry 2005).

Ticks and Tick-borne Pathogens

Pathogens vectored by ticks are considered a significant public health risk. Incidence of tick-borne diseases worldwide has increased in the last several decades and several new diseases have emerged (Maeda et al. 1987, Parola et al. 2005, Goodin et al. 2006, Apperson et al. 2008). For example, in the United States, cases of Lyme disease, ehrlichiosis, and Rocky Mountain spotted fever (RMSF) have increased greatly in the past 30 years (Fishbein et al. 1984, White et al. 1991, Dalton et al. 1995, Orloski et al. 2000, Openshaw et al. 2010). Furthermore, diseases such as Southern Tick-Associated Rash Illness (STARI), American boutonneuse fever, and babesiosis (not caused by *Babesia microti*) have emerged in the last 10-15 years (Masters et al. 1998, James et al. 2001, Paddock et al. 2004, Chauvin et al. 2009). While a number of factors are believed to contribute to this trend, it is generally believed that the primary drivers are changes in human land-use and management practices (Allan et al. 2003, Patz et al. 2005, Goodin et al. 2006). Climate change is also predicted to alter the ecology of vector-borne zoonoses due to changes in vector life cycles and distributions (Dobson and Carper 1992, Martens et al. 1997, Harvell et al. 2002, Patz et al. 2005). Therefore, to manage tick-borne diseases and decrease infection risk, it is imperative that we understand current vector distributions and population dynamics and how human land management practices, such as prescribed burns, may affect tick populations and tick-borne pathogen prevalence.

There are several species of ticks in Georgia capable of transmitting tick-borne pathogens to people. The most common ticks in Georgia associated with pathogen transmission (in descending order of abundance) are the lone star tick (*Amblyomma americanum*), American dog tick (*Dermacentor variabilis*), black-legged tick (*Ixodes*

scapularis), and the Gulf Coast tick (*A. maculatum*) (Georgia Department of Human Resources 2006). All of these tick species are capable of transmitting one or more zoonotic pathogens (Table 1). While the vectors of most tick-borne pathogens are generally known and accepted, there are some cases in which the causative agent of a particular disease is unclear and/or the capability of alternative tick vector species to maintain and transmit a pathogen is poorly understood. For example, *A. americanum* is associated with the newly emerging STARI but a causative agent hasn't been identified. While there evidence is that *Borrelia lonestari* or *Rickettsia amblyommii* may be a causative agent of STARI, neither species has been definitively identified as the agent. Specifically, James et al. (2001) detected *B. lonestari* DNA in an attached tick and skin biopsy of a presumed STARI patient. However, subsequent studies have not been able to detect *B. lonestari* in STARI patients (Wormser et al. 2005, Masters et al. 2008). More recently, serologic evidence of *Rickettsia amblyommii* has been detected in some STARI patients, but definitive evidence is lacking (Billeter et al. 2007, Apperson et al. 2008, Parola et al. 2009, Nicholson et al. 2009).

Prevalence of tick-borne pathogens varies spatially and temporally. For example, a study in northeastern Georgia found a variable difference in prevalence based on year with *Ehrlichia* prevalence ranging from 6.0% to 10.0% and *B. lonestari* prevalence ranging from 0-4.0% (Varela et al. 2004). Additionally, Mixson et al. (2006) tested the prevalence of various tick-borne pathogens across 29 sites in nine states and found that the prevalence of *E. chaffeensis* in *A. americanum* ranged between 0 and 27% with large differences based on site. Because tick populations and associated pathogens can vary, there is a need to understand tick population dynamics within a particular region. Such

knowledge provides valuable insight into potential threats to public health that could not necessarily be inferred by knowledge based on data from other regions. Furthermore, monitoring for multiple years is valuable to detect changes in tick dynamics that occur on an annual basis due to climate differences.

Table 1.1. Common human-biting ticks of Georgia and their associated pathogens and human diseases. Note that the ticks are listed in order from most to least common.

Common Name of Tick	Scientific Name	Pathogens Vectored	Associated Disease
lone star tick	*Amblyomma americanum*	*Ehrlichia chaffeensis*	Human Monocytic Ehrlichiosis (HME)
		Ehrlichia ewingii	Ehrlichiosis *ewingii*
		causative agent of STARI (*Borrelia lonestari*?)	Southern Tick-Associated Rash Illness (STARI)
American dog tick	*Dermacentor variabilis*	*Rickettsia rickettsii*	Rocky Mountain spotted fever (RMSF)
black-legged tick	*Ixodes scapularis*	*Borrelia burgdorferi*	Lyme disease
		Anaplasma phagocytophilum	Human Granulocytic Anaplasmosis (HGA)
		Babesia microti	Human Babesiosis
Gulf Coast tick	*Amblyomma maculatum*	*Rickettsia parkeri*	American boutonneuse fever

In Georgia, several tick-borne diseases are reportable; however, many tick-borne diseases are under-reported due to patients never seeking medical attention or being

incorrectly diagnosed. Regardless, in 2007, 60 clinical cases of RMSF, 14 cases of ehrlichiosis (human monocytic ehrlichiosis [HME] and human granulocytic ehrlichiosis [HGA]), 11 cases of Lyme disease, and 2 cases of Q fever (*Coxiella burnettii*) were reported (Garrison 2008). Although STARI is not reportable due to the lack of knowledge regarding its etiology, a number of suspected cases have occurred in Georgia (Felz et al. 1999).

Tick activity varies seasonally based off of the tick species and life stage. Therefore, transmission and disease risk also varies seasonally based on individual tick life cycles (Lindsay et al. 1999, Goodwin et al. 2001). This seasonality is also important when predicting how various management plans may affect tick populations. All tick species capable of transmitting zoonotic pathogens in Georgia are three-host Ixodid (hard) ticks. Three-host Ixodid ticks typically require 1-2 years to complete their lifecycle (Figure 1). The specific months that each life stage emerges and peaks in abundance varies regionally and by tick species. For example, Cilek and Olson (2000) reported that adult *A. americanum* activity peaked in May in northwestern Florida. Conversely, Cully (1999) found that adult *A. americanum* activity did not peak until late June in Kansas. Thus, understanding when various tick species and life stages are active within a region is important for public health as it indicates when risk of transmission of tick-borne pathogens may occur.

To date, several studies have examined the seasonality of tick species in north-central Georgia and northern Florida (Davidson et al. 1994, Cilek and Olson 2000, Clark 2004); however, no studies have been conducted in southwestern Georgia, which has unique land cover and geography with large tracts of agriculture and pine or mixed pine

forests. Additionally, the predominant soil type is sandy which is very different from the Piedmont region of Georgia. These factors directly impact patch connectivity, host abundance, vegetation and microclimate, all of which affect tick species abundance

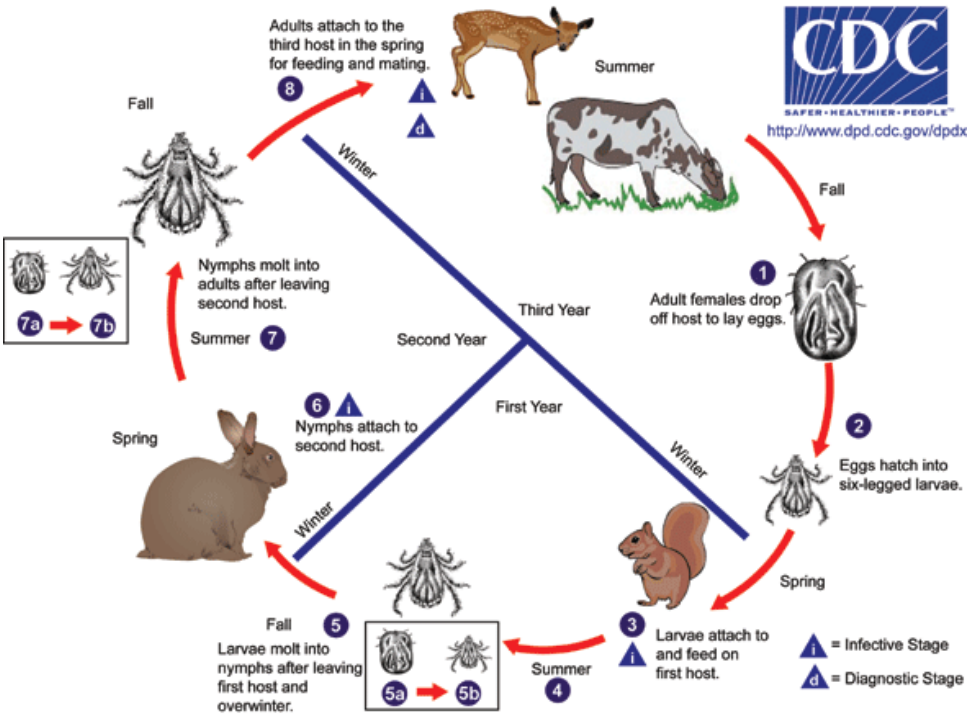

Figure 1.1. General life cycle of three-host Ixodid (hard) ticks. Obtained from www.cdc.gov.

and composition (Ostfeld et al. 1995, Estrada-Peña 1998, Schulze et al 2002, Perret 2004). Thus, it seems probable that tick dynamics in the southwestern part of Georgia may differ from those in other regions of Georgia or Florida.

Host Preferences of Ticks

Host diversity and abundance are important factors when attempting to understand tick diversity and abundance. Certain species of ticks have host species preferences and because ticks are passive dispersers, they rely on their hosts to disperse

and travel long distances (Falco and Fish 1991, Estrada-Peña 2003). Additionally, vertebrate hosts are often the reservoirs or amplifying hosts for the pathogens that cause disease in humans and domestic animals. Importantly, a number of wildlife species that are important hosts to ticks or reservoirs of tick-borne zoonotic pathogens have experienced explosive population growth over the past several decades including white-tailed deer (*Odocoileus virginianus*), coyotes (*Canis latrans*), and turkeys (*Meleagris gallopavo*) (Childs and Paddock 2003, Paddock and Yabsley 2007, Thompson et al. 2009). As urban areas continue to expand and encroach into habitats of these vectors and hosts, the risk for transmission of tick-borne disease to humans increases (Goodin et al. 2006, Paddock and Yabsley 2007, Thompson et al. 2009).

Ticks generally have host preferences which vary by species and life-stage. These preferences are an integral part of tick population dynamics, as host preferences affect tick distributions as well as pathogen presence and dispersal. For example, *A. americanum* are generalists that typically show little preference for hosts, however, all life-stages are commonly found on white-tailed deer an important reservoir of *E. chaffeensis* and *E. ewingii* (Tugwell and Lancaster 1962, Clymer et al. 1970). In contrast, *I. scapularis* larvae and nymphs are primarily found on rodents, especially the white-footed mouse (*Peromyscus leucopus*), and to a lesser extent on mesomammals, ground-dwelling birds and lizards (Anderson and Magnarelli 1984, Levine et al. 1985, Battaly et al. 1987, Mannelli et al. 1993, Levine et al. 1997), while adults feed primarily on white-tailed deer (James and James 1990, Clark et al. 2001, Schulze et al. 2005).

Perhaps the best-studied example of how host composition can affect disease risk is Lyme disease and the dilution effect. In the northeastern US, the white-footed mouse is

the primary reservoir of *Borrelia burgdorferi*, the causative agent of Lyme disease (Levine et al. 1985, Donahue et al. 1987, Bengis 2004); however, other small mammals such as chipmunks, meadow voles and shrews are also competent alternative reservoirs (Mather at al. 1989, Telford et al. 1990, Markowski et al. 1998, Brisson et al. 2008). Conversely, there are a number of hosts that are poor or incompetent reservoirs which are known as "dilution hosts" because ticks feed on these hosts yet do not become infected with *Borrelia*. These hosts include white-tailed deer, Eastern grey squirrels (*Sciurus carolinensis*) and the Virginia opossum (*Didelphis virginiana*) (Fish and Daniels 1990, LoGuidice et al. 2003). Interestingly, this theory describes how as biodiversity is lost due to habitat fragmentation, generalist species such as the white-footed mouse will increase which leads to increased numbers of *Borrelia*-infected ticks (Ostfeld and Keesing 2000, Schmidt and Ostfeld 2001). Because many generalist species are competent reservoirs, pathogen prevalences are generally higher in smaller forest patches where biodiversity is lower (Ostfeld et al. 2000, Allan et al. 2003, LoGiudice et al. 2003). The dilution theory has become a well-established theory in disease ecology and studies are underway to determine if a similar mechanism is important in the ecology of other diseases such as West Nile virus, malaria and schistosomiasis (Swaddle and Carlos 2008, Pongsiri et al. 2009).

General Factors Associated with Tick Abundance

There are numerous factors that influence tick occurrence and abundance both at the macro- and micro-scale (Buskirk and Ostfeld 1998, Lindsay et al. 1999). Ticks are particularly susceptible to desiccation and therefore survive and/or become active only

when their microhabitat has the appropriate temperature and humidity (Ostfeld et al. 1995, Schulze et al. 2002, Perret 2004). Vegetation can alter microhabitat and so can be important for ticks (Duffy et al. 1994b, Ostfeld et al. 1995, Estrada-Peña 1998, Schulze et al 2002, Perret 2004). For example, wooded areas with high shrub density and thick leaf litter exhibit higher *A. americanum* abundances than more open areas with little vegetation or ground litter (Schulze et al. 1998, Schulze et al. 2002).

Because certain tick species prefer wooded habitats with abundant ground litter, it is suspected that fire-dominated ecosystems may create a habitat that is less hospitable to certain tick species. The use of fire is known to strongly influence ecosystem structure (Rego et al. 1991, Brockway and Lewis 1997, Bond and Keeley 2005). Specifically, regularly burned longleaf pine forests of the Lower Coastal Plain tend to have semi-open canopies and dense under-stories, but minimal ground litter. This presumably leaves ticks more susceptible to desiccation as ground litter is thin and sunlight more readily penetrates through the canopy.

In addition to microhabitat, host abundance is also thought to be a predictor of tick abundance (Estrada-Peña 2003) but results of studies are contradictory. Many studies have failed to link a current year's host abundance with tick abundance (Lindsay et al. 1999, Goodwin et al. 2001, Schulze 2001, Jordan and Schulze 2005), but other studies have found support for this theory (Duffy et al. 1994a). These conflicting results may be a result of factors that were not accounted for such as habitat differences or microclimate. Furthermore, it is unclear how long it takes a tick population to change in response to an increase or decrease in its host population. Interestingly, there is evidence that the previous year's host abundance and host food resources in an area may be more

indicative of a current year's abundance of ticks (Ostfeld et al. 2006). These findings are presumably due to the ticks' lifecycle, which spans approximately 2 years. Additional evidence that host abundance may influence tick abundance has been obtained through host exclusion studies (Bloemer et al. 1986, Daniels et al. 1993, Stafford 1993). Daniels et al. (1993) found that in areas where deer had been excluded for 25 years, there were 83% fewer nymphs and 90% fewer larvae as compared to areas where deer were not excluded. Another study found that an 83% reduction of deer abundance in an area lead to a nearly 50% reduction in *Ixodes scapularis* (Deblinger et al. 1993). Thus, when making predictions regarding tick abundance, one must integrate knowledge of the previous year's food resources and host abundance, as well as the current state of the microhabitat.

How Prescribed Fire Affects Ticks

When attempting to understand how regular prescribed burns will affect ticks and ultimately, human disease risk, it is important to understand how fire affects ticks, hosts, and the microhabitat. It is generally agreed upon that fire results in an immediate decrease in ticks and that tick populations recover after a burn (Hoch et al. 1972, Jacobson and Hurst 1979, Drew et al. 1985, Wilson 1986, Davidson et al. 1994, Stafford et al. 1998, Cully 1999). However, the long-term effects of prescribed fire on tick populations are not fully understood. For example, many studies have found that fire causes a long-term decrease in tick populations and in some cases pathogen prevalence as well (Hoch et al. 1972, Jacobson and Hurst 1979, Drew et al. 1985, Wilson 1986, Stafford et al. 1998, Cully 1999). Indeed, Jacobson and Hurst (1979) evaluated tick

parasitism of eastern wild turkeys (*Meleagris gallopavo*) released into burned and unburned plots and found significantly fewer ticks on turkeys in burned plots. Furthermore, Stafford et al. (1998) found a lower percentage of ticks infected with *B. burgdorferi* in burned areas. However, other studies have found an increase in tick populations following a burn (Mather et al. 1993, Cilek and Olson 2000) or that populations return to their pre-burn levels within a year (Davidson et al. 1994, Stafford et al. 1998, Cully 1999). Rapid return of ticks could be due to enhanced wildlife use after burns due to lush re-growth that occurs immediately after a fire. For example, Main and Richardson (2002) compared white-tailed deer use of study plots before and after fire and found that use by white-tailed deer was significantly greater up to 6 months after a fire. Similarly, Edwards et al. (2004) observed that deer usage of plots that had been treated with a combination of herbicide, fire and fertilizer, increased after treatments. Indeed, deer's preference for cleared areas or edge habitat, versus dense, wooded areas has been confirmed in other studies as well (Johnson et al. 1995, Jordan and Schulze 2005). Main and Richardson (2002) found that when plots burned at different times were compared, those that had been burned more recently were used more by white-tailed deer than those in which more time had passed since a burn.

Perhaps contributing to the discrepancies in the literature is that many of these studies did not take into account host abundance, microclimate and vegetation structure. Furthermore, these studies often failed to simulate real-world management conditions in that study burns were only performed singularly, over a small area of land and/or were performed in areas that were previously unburned. This is in contrast to management

situations which typically perform burns over hundreds to thousands of acres on a regular basis.

Nevertheless, it is generally agreed upon that certain factors related to burning such as burn intensity and interval may play an important role in the long-term impact on a tick population. For example, the extent of the initial decrease of a tick population after a burn has been found to be correlated to the intensity of the burn, with higher intensity burns correlating to the amount of ground litter destroyed (Stafford et al. 1998). Furthermore, time interval is believed to potentially play an important role in the impact that long-term prescribed burning may have on tick populations. Cully (1999) compared tick abundance between sites burned every 1, 4 and 20 years, and found that sites burned at 1-year intervals had fewer ticks than those burned every 4 and 20 years, with no difference between the 4-year and 20-year intervals. Furthermore, it was found that any site burned within the year had fewer ticks than any site burned 1 or more years previously. Davidson et al. (1994) also compared annual versus biennial burns, and found that both reduced tick abundance; however, over a 5-year period, the degree to which ticks were reduced with biennial burns was less than that seen with annual burns. Thus, it appears that annual, and even biennial burns to a lesser extent, may affect tick populations on a long-term basis due to the inability of the population to fully re-establish after fire. However, less frequent burns (every 3 years or more), may only affect tick populations on a short-term basis (i.e., burn year).

Red imported fire ants (RIFA) and their impacts on ticks

Red imported fire ants (*Solenopsis invicta*) (RIFA) were first introduced into the United States around 1940 in Mobile Bay, Alabama (Tschinkel 1993). It is presumed that the initial introduction was via a ship from Brazil where they are native. Regardless of the route of introduction, RIFA rapidly established and expanded throughout a large portion of the Southeast through both natural and human-assisted dispersal. Red imported fire ants prefer sites that receive direct sunlight and are often associated with disturbed habitat (Anonymous 1958, Hung and Vinson 1978, Howard and Oliver 1979, Tschinkel 1986, Jemal and Hugh-Jones 1993). Indeed, RIFA is very common in burned and/or open habitats and is less common in fully forested, unburned habitat (Anonymous 1958, Green 1967, Fleetwood et al. 1984).

Red imported fire ants are considered a nuisance due to their aggressive response to mound disturbance and painful bite which can rarely require medical attention or cause death (Adams and Lofgren 1981, Rhoades et al. 1989, Jemal and Hugh-Jones 1993). Red imported fire ants also impact agriculture as it can damage agricultural crops and occasionally has been implicated in the destruction of agricultural machinery (Burns and Melancon 1977, Glancey et al. 1979, Apperson and Powell 1983, Tschinkel 1993). Interestingly, however, some entomologists have argued that RIFA can be beneficial to agricultural systems as they can reduce a number of agricultural pests (e.g., the sugarcane borer (*Diatraea saccharalis*)) (Long et al. 1958, Hensley et al. 1961, Negm and Hensley 1967, Negm and Hensley 1969).

There have been a few documented instances of RIFA reducing lone star tick populations under experimental and non-experimental field settings (Harris and Burns

1972, Burns and Melancon 1977, Fleetwood et al. 1984). For example, Harris and Burns (1972) found that nymphal and adult lone star ticks released into sites with RIFA had significantly lower survival than when released into sites that had been treated with mirex bait to eliminate RIFA. Furthermore, Burns and Melancon (1977) found in a before-after control-impact (BACI) design that when RIFA were treated with mirax bait, tick abundance was proportionally greater than in sites at which bait had not been used. Predation of ticks by ants was also noted in Guadeloupe, French West Indies where tropical fire ants (*Solenopsis geminate*) were the primary predator of the tropical bont tick (*Amblyomma variegatum*) (Barré et al. 1991).

While there is not enough data to definitively state the impacts of RIFA on tick populations (especially tick species other than the lone star tick), it does appear that RIFA may be an important predator of ticks in the Southeast. Corroborating this idea is an interesting study performed by Yoder et al. (1992) which found that metastriate ticks, such as *Amblyomma* spp., produce an ant-diversionary secretion from their large wax glands. These secretions resulted in the reduction of predation on ticks by predatory ants such as RIFA. Such an adaptation would seem to indicate the possibility that predatory ants are acting as an important selective force on metastriate ticks.

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CHAPTER 3

THE EFFECTS OF LONG-TERM PRESCRIBED BURNING ON TICK POPULATION DYNAMICS IN SOUTHWESTERN GEORGIA*

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Abstract

Tick populations have increased dramatically in the past several decades leading to an increase in the incidence and emergence of tick-borne diseases throughout the United States. Methods that can effectively reduce tick populations and tick-borne disease risk are needed. One promising method is prescribed fire; however, the efficacy of prescribed burning as a mechanism for tick control is unclear because past studies have provided conflicting data, likely due to a failure to simulate operational management scenarios and/or account for other predictors of tick abundance. Therefore, our study was conducted to increase knowledge of tick population dynamics relative to long-term prescribed fire management. Twenty-one sites in southwestern Georgia with varying burn regimes (burned surrounded by burned [BB], burned surrounded by unburned [BUB], unburned surrounded by burned [UBB], and unburned surrounded by unburned [UBUB]) were sampled monthly for 2 years while simultaneously collecting data on variables that can affect tick abundance (e.g., host abundance, vegetation structure, and micro- and macro-climatic conditions). In total, 47,185 ticks were collected, of which, 99% were *Amblyomma americanum* followed by *Ixodes scapularis* (0.7%) and fewer numbers of *A. maculatum*, 1 *I. brunneus*, and *Dermacentor variabilis.* Monthly seasonality trends were similar between 2010 and 2011. Long-term prescribed burning consistently and significantly reduced tick counts (overall as well as specifically for *A. americanum* and *I. scapularis)* regardless of the burn regimes evaluated and all other variables evaluated. Interestingly, tick species composition varied according to burn regime with *A. americanum* dominating collections at UBUB sites, *A. maculatum* at BB sites, *I. scapularis* at UBB sites, and a more even composition at BUB sites. These data indicate

that regular prescribed burning is an effective tool for reducing tick populations and ultimately may reduce risk of tick-borne disease.

Introduction

In the past several decades, numerous novel tick-borne diseases have emerged and the incidence of other tick-borne diseases has increased (Maeda et al., 1987; Parola et al., 2005; Goodin et al., 2006; Apperson et al. 2008; Bacon et al., 2008; Ogden et al., 2009; Thomas et al., 2009). While this increase is likely due to a number of factors, such as increases in reporting, diagnosis and host abundance, one of the primary drivers is thought to be human land modification and management practices (Allan et al. 2003; Patz et al., 2005; Goodin et al., 2006). This hypothesis has underscored the importance of understanding how human land management practices affect tick population dynamics as well as identifying methods to control tick populations and reduce human disease risk.

Perhaps one of the most promising methods for controlling tick populations is prescribed fire because it can be applied on a landscape level and is relatively time and cost efficient. Additionally, prescribed fire is a well-accepted form of ecosystem management and wildfire prevention (Haines et al., 2001; Kirkman et al., 2001; Fernandes and Botehlo, 2003). In the Southeast, the value of prescribed fire is particularly apparent where forests are dominated by fire-adapted and –dependent tree species, such as longleaf pines (*Pinus palustris*). Specifically, fires reduce fuel load and cause regeneration and diversification of the understory of forests (Towne & Owensby, 1984; Rego et al., 1991; Sparks et al., 1998; Kirkman et al., 2001; Knapp et al., 2005). In addition, the immediate regeneration of understory vegetation after a burn and the long-

term density and diversity of understory vegetation within regularly burned habitat provides resources for many wildlife species including the endangered or threatened gopher tortoise (*Gopherus polyphemus*), Eastern indigo snake (*Drymarchon couperi*), and Red-cockaded woodpecker (*Picoides borealis)* (Hobbs and Stowart, 1984; Sparks et al., 1998; Kirkman et al., 2001, Allan 2009).

Several past studies have been conducted on the impacts of prescribed fires on tick populations. Although there have been conflicting results, the majority of studies concluded that tick populations are reduced immediately after a fire event, but recover to pre-burn abundance within 1 year (Hoch et al., 1972; Jacobson & Hurst, 1979; Drew et al., 1985; Wilson, 1986; Mather et al., 1993; Davidson et al., 1994; Stafford et al., 1998; Cully, 1999; Cilek and Olson, 2000). However, others failed to see a significant reduction in ticks and some even saw an increase (Padgett et al. 2009, Polito 2010,Willis et al. 2012). However, many previous studies did not simulate real-world management conditions in that burns in most studies were performed only once, over a small area of land and/or were performed in areas that were previously unburned (Hoch et al. 1972, Jacobson and Hurst 1979, Drew et al. 1985, Mather et al. 1993, Davidson et al. 1994, Cully 1999, Bailey and Whitham 2002, Frater 2011). This is in direct contrast to the typical regimen for applying prescribed burns, which are conducted over hundreds to thousands of acres on a regular basis. These discrepancies have clear implications for tick re-establishment after a fire and should be accounted for in an analysis on the impacts of fire on tick populations. Furthermore, previous studies typically did not investigate other factors known to affect tick populations such as host abundance, microclimate and

vegetation structure (Drew et al. 1985, Davidson et al.1994). Failure to account for such factors may explain some of the conflicting results of past studies.

To address impacts of long-term prescribed burning on tick population dynamics and evaluate how prescribed fire might interact with other factors to affect tick abundance, we determined tick abundance, species composition, and seasonality at multiple sites with variable burn histories with >10 years of operational burn management. Additionally, other factors known to potentially affect tick abundance (e.g., host abundance, vegetation structure and microclimate) were evaluated at each site. Collectively, this data will provide insight in to the efficacy of long-term prescribed burning for tick control and also reveal phenologies for numerous tick species in southwestern Georgia, a region for which there is currently little to no data on ticks or tick-borne pathogens.

Methods

Study Area

This study was conducted in southwestern Georgia, which is dominated by pine, mixed pine forests and agriculture. Prescribed burning is commonly used throughout the region to maintain various pine ecosystems including, longleaf pine ecosystems. We selected 21 sites based on the presence or absence of a long-term history of prescribed burning, with burned sites having had at least 10 years of regular prescribed burns and unburned sites having no history of prescribed fire for > 10 years (Table 3.1). To account for differences in land cover and management both within the site and immediately surrounding the site, each site was further categorized as being 1) burned surrounded by

burned (BB), 2) burned surrounded by unburned (BUB), 3) unburned surrounded by burned (UBB) and 4) unburned surrounded by unburned (UBUB) (i.e., a control).

Tick Collections

Each site was sampled monthly for ticks for 24 months (January 2010-December 2011) using 1m x 1m flags made of flannel cloth (Zeman 1997). To standardize conditions, flagging was conducted only when the temperature was above 7.2°C and after 10AM when vegetation was dry (no dew or moisture from precipitation). Additionally, flagging was not conducted during inclement weather (rain, snow, or excessive wind). Flagging time was standardized, with a minimum of 1 hour per site per month being performed. Flags were checked every 10 minutes and all nymphs and adults were collected and preserved in 70% ethanol. All larvae were removed en masse with masking tape with each clutch being kept separate.

Tick Identifications

All adult and nymphal *Amblyomma* spp. and adult *Dermacentor variabilis* (Acari: Ixodidae, Say), and *Ixodes* were identified morphologically utilizing a microscope and key (Kierans and Litwak, 1989; Kierans and Durden 1998). All larvae, *Ixodes* spp. nymphs, and a subset of adult *Ixodes* spp. were confirmed through amplification and sequence analysis. DNA was extracted from ticks using a DNeasy blood and tissue kit (QIAGEN, Valencia, CA) utilizing the manufacturer's protocol. For *Amblyomma* larvae collected in 2010, a multiplex real-time polymerase chain reaction (qPCR) was used to differentiate between *A. maculatum* and *A. americanum* as described by Zemtsova at al. (2013). For *Amblyomma* larvae collected in 2011 and all *Ixodes*, a PCR targeting the 16S rDNA gene using primers 16S-1 (5'-CCGGTCTGAACTCAGATCAAGT) and 16S+2

(5'-TTGGGCAAGAAGACCCTATGAA) was used as described in Black and Piesman (1994). DNA extraction and PCR reactions were performed in hoods dedicated strictly for their respective tasks and waters were included as negative controls to detect contamination. All amplicons from the 16S protocol were bi-directionally sequenced at the Georgia Genomics Facility (Athens, GA).

Host Monitoring

Host presence and relative usage of each site was determined through quarterly trail camera (Cuddeback Capture, Green Bay, WI) surveys. For each survey, one camera was placed at each site on an unbaited, secondary dirt road or trail bordering or within 100 m of the site. All sites were surveyed during an 11-day period (with the exception of the winter 2011 survey in which all sites were surveyed within 30 days due to logistical constraints) with each survey lasting 76 hours. The species, date, and time of all animals captured were recorded.

Vegetation Surveys

To account for potential differences in vegetation among the sites, vegetation surveys were performed at all sites. To determine tree density, a singular point-centered quarter survey utilizing the equivalent of 20 points per hectare was performed as described by Mitchell (2007) with the following exceptions: 1) points at which surveys were performed were randomly generated, with each point being $\geq$ 20 m from any other point, 2) trees were identified as pine or hardwood and deciduous or non-deciduous, and 3) a quarter was considered empty if there were no trees within 10 m from the center point. Selected points were marked for subsequent surveys.

Understory vegetation and canopy closure was measured using quarterly surveys at points used for the point-centered quarter survey. To perform the survey, a 1m x 1m frame was placed with the point falling at the center of the frame and the sides of the frame running directly north to south and east to west. Percent cover and composition of understory vegetation and ground litter was estimated. Litter depth was measured at two random points within the frame. Canopy cover was measured from the center point using a spherical densiometer.

Microclimate and Weather

At least three microclimatic measurements (humidity, temperature, and median wind speed) were taken at each site immediately after each monthly flagging using a Kestrel 3000 Weather Meter (Birmingham, MI). Measurements were taken at ground level and 1 m above the ground. If a particular site had areas with ticks and other areas without ticks, measurements were taken where ticks were collected and two additional measurements were taken at randomly assigned points where ticks were not detected. If no ticks were collected at a site, measurements were taken at randomly selected points.

Weather data including maximum temperature and precipitation the current sampling day, maximum and minimum temperatures the previous day, and precipitation accumulation the previous 3 days were collected for all sites, each sample date from the JERC Weather Station (all JERC locations) and the National Weather Service (all other sites).

Statistical Analyses

A generalized estimating equation (GEE) negative binomial regression model was used (STATA 12.1 2011) to evaluate the impacts of long-term prescribed burning, host

abundance, vegetation composition, microclimate, and weather on tick abundance. This GEE model was used because it is well-suited for repeated measures, accounts for clustering by site, and is appropriate for use when there are large numbers of zeros in the data. Models were created for 1) total tick counts, 2) *A. americanum* counts, and 3) *I. scapularis* counts. When applicable, each larval cluster was counted as a single tick so as not to inadvertently skew data. No other tick species were abundant enough for further analysis.

Because host and vegetation surveys were only conducted quarterly, all data were evaluated on a quarterly basis (spring, summer, fall, and winter). Thus, for tick counts, microclimate, and weather data, we used data that were collected closest to the date of the vegetation surveys for each quarter. Linearity of all variables was tested and variables converted to categorical data if non-linear.

For each model, all predictor variables associated with tick counts were analyzed using a univariable GEE analysis, and variables with $p < 0.20$ were selected for inclusion in the multivariable models. It was decided that year and season variables would be retained in the multivariable model regardless of their respective p-values because it is generally accepted that these variables would be important factors to account for in evaluating tick abundance. Calendar seasons were consolidated into warm (i.e., spring and summer) and cold (fall and winter) seasons because they were not statistically significant from one another regarding tick counts in initial GEE models. Remaining variables were removed from the multivariable model until only those with $p < 0.05$ remained. Upon reaching a preliminary model, the significance of all removed variables

was re-evaluated in the simplified model and all two-way interactions with burning were evaluated.

Results

In total, 47,184 ticks were collected with *A. americanum* being the most common tick detected followed by *I. scapularis, A. maculatum, D. variabilis,* and *I. brunneus* (Tables 3.2-3.5). To account for varying effort among sites and collection dates, we standardized by evaluating ticks collected/hr. Seasonality trends were determined using all 21 sites for both 2010 and 2011 (Figure 1). In 2010, *A. americanum* activity peaked in May for adults, May and October for nymphs, and July and September for larvae. In 2011, periods of peak *A. americanum* activity were similar with adults peaking in April through June, nymphs in May and September, and larvae in July and October. Adult *A. maculatum* peaked June through August in 2010 and in August in 2011 (Figure 3.1). A single *A. maculatum* nymph was collected in April of 2010 and in March of 2011 and a single *A. maculatum* larval clutch was collected in September of 2011. *I. scapularis* adults peaked in November and March of 2010 and in February in 2011. *D. variabilis* adults peaked in July in 2010 and were not caught in enough abundance in 2011 to determine seasonality.

Within burn treatments, 43 ticks were collected in BB sites (1 *A. americanum*, 37 *A. maculatum*, and 5 *I. scapularis*), 1,756 in BUB sites (1,687 *A. americanum*, 32 *A. maculatum*, 26 *I. scapularis*, and 11 *D. variabilis*), 3,719 in UBB sites (3,568 *A. americanum*, 6 *A. maculatum*, 135 *I. scapularis*, 1 *I. brunneus*, and 9 *D, variabilis*), and 41,706 in UBUB sites (a minimum of 41,460 *A. americanum*, a minimum of 4 *A.*

maculatum, 186 *I. scapularis*, and 18 *D. variabilis*). Our modeling efforts suggested that total tick counts were affected by long-term burning, season, percent litter cover, and trees per hectare (Table 3.6). There was a significant interaction between burning and season. For sites in which burning occurred, tick counts did not change significantly by season [RR = 1.1 (95% CI: 0.57, 2.12); P = 0.774], confirming the trend that ticks were significantly reduced in burned sites (Figure 3.2). However, at sites in which there was no burning, tick counts were 10 times greater in the warm season than in the cold [Relative Risk (RR) = 10.7 (95% CI: 4.20, 27.18); P <0.001]. Having higher than 95% litter cover was positively associated with an approximately two-fold increase in tick counts and density of trees (> 183 per ha) was associated with an approximately six-fold increase in tick counts.

Interestingly, the different burn categories were dominated by different tick species, with *A. americanum* being the most prevalent tick in UBUB sites, *A. maculatum* being most common in BB sites, *I. scapularis* dominating UBB sites, and a more even distribution of the different tick species at BUB sites (Table 3.1; Figure 3.3). To further investigate impacts of the measured variables on individual tick species, we constructed models specifically for *A. americanum* (Table 3.7) and *I. scapularis* (Table 3.8). *A. americanum* counts were best predicted by burning, season, bobcat presence/ absence, and wind at ground level on day of tick counts. A significant interaction occurred between burning and season, with *A. americanum* not being significantly affected by season in burned sites [RR = 2.4 (95% CI: 0.3, 19.0); P = 0.409]. However, at sites in which there was no burning, *A, americanum* counts were over 30 times greater in the warm season than in the cold [RR = 33 (95% CI: 14, 81); P <0.001], suggesting that

prescribed burning reduced *A. americanum* abundance. The presence of bobcats (*Lynx rufus*) was associated with a significant increase in *A. americanum* counts, while an increase in wind by 1 mile per hour resulted in an 85% decrease in *A. americanum* detectability. Finally, *I. scapularis* counts were best predicted by burning, season, year, trees per hectare, and precipitation. Long-term prescribed burning reduced *I. scapularis* counts by 78%. Furthermore, *I. scapularis* counts were reduced by approximately 85% in the warm season relative to the cold season and was 3.7 times higher counts in 2011 than in 2010. Lastly, high tree density (>183 trees per ha) resulted in a nearly 18 fold increase in *I. scapularis* and precipitation occurring 3 days prior to sampling resulted in a two-fold increase in counts.

Discussion

To our knowledge, this is one of the most comprehensive studies performed on the effects of long-term operational prescribed burning while also accounting for other variables known to affect tick abundance including host abundance, vegetation structure, microclimate, and weather. Furthermore, this is the first long-term, study of tick phenology in southwestern Georgia, and provides valuable insight into seasonality and species composition within common habitats of the region.

Overall, gaining a better understanding of tick species composition and seasonality in this rarely studied region provides important information for public health education and research. Our findings regarding seasonality of ticks were generally in agreement with studies done in other regions of Georgia and northern Florida (Davidson et al. 1994, Cilek and Olson 2000). One exception was that, similarly to Cilek and Olson

(2000) whom worked in northern Florida, we detected a bi-modal peak for *A. americanum* nymphs in both years which was in contrast to the single peak noted in Davdison et al. (1994) in the Piedmont region of Georgia (i.e., farther north). Thus, nymphal *A. americanum* populations south of the fall line appear to have bi-modal peaks whereas those north of the fall line have a single peak. Also, to our knowledge, this marks only the second longitudinal study on *A. maculatum* seasonality (Goddard and Paddock 2005), providing important information on this tick which has gained interest in recent years having been identified as the vector of the newly emerging American boutonneuse fever (Paddock et al. 2004).

Past studies suggested that both weather and rainfall affect tick abundance and distribution (Davidson et al. 1994, Ogden et al. 2006) and we suspect that the differences observed in tick phenology from 2010 to 2011 were a result of weather. In 2011 there were fewer *A. americanum* and *A. maculatum* (although not significantly so) and significantly more *I. scapularis.* Furthermore, the bi-modal beak of *I. scapularis* was later in 2011 as compared to 2010. These differences may have been due to an exceptionally dry summer (the peak of *A. americanum* and *A. maculatum* activity) followed by a wet fall and mild winter (*I. scapularis* adult peak activity) (data not shown) in 2011 as compared to 2010.

Long-term prescribed burning significantly reduced tick counts. Although some previous studies have found either increases in tick populations or a recovery of tick populations after a period following a burn (Hoch et al., 1972; Jacobson & Hurst, 1979; Drew et al., 1985; Wilson, 1986; Mather et al., 1993; Davidson et al., 1994; Stafford et al., 1998; Cully, 1999; Cilek and Olson, 2000, Padgett et al. 2009), our results suggest

that prior conclusions may be the result of failure to account for other variables and failure to simulate real-world management scenarios in which large-scale burns are performed across the landscape on a regular basis. Because operational burns typically occur on larger areas, re-colonization by ticks may take longer than re-colonization of smaller-scale burns like those used in some prior studies. Furthermore, repeated burnings over a long period of time may result in depletion of source populations of ticks.

Interestingly, we observed variation in the tick community composition depending on the burn treatment which, to our knowledge, has never been reported. The most striking difference was related to higher abundances of *A. maculatum* being found in BB sites as compared to *A. americanum* and *A. americanum* being most abundant in UBUB sites. It is generally known that *A. americanum* prefer hardwood forests (Semtner et al. 1971) and there have even been some suggestions that *A. americanum* outcompetes other tick species such as *D. variabilis* (Stromdahl and Hickling 2012). Thus, the dominance of *A. americanum* in UBUB sites would arguably be expected. The greater question, of course, is why the absence of *A. americanum* and dominance of *A. maculatum* in BB sites? Burning at BB sites was used to directly alter the physical vegetation structure and maintain the pine ecosystem. Thus, BB sites were generally long-leaf pine forests with a diverse understory, lack of mid-story, and a semi-open pine canopy. These vegetation conditions lead to a hotter, dryer environment and we suspect that this plays a key role in both the long-term reduction of ticks and shift in tick species composition observed at these sites. Our models support this idea as vegetation structure (tree density and litter cover specifically) and microclimatic variables which would affect tick moisture retention were most important in predicting tick counts. Willis et al. (2012)

had similar findings in that *A. americanum* was found to be associated with litter cover and negatively associated with pine sapling density. Also in support of this theory, Gleim et al. (2013) reported that survival of *A. americanum* had significantly lower survival in experimental enclosures in burned habitats vs. unburned habitats compared with *A. maculatum*. Collectively, these data suggest that the ticks' different physiologic and/or behavioral traits allow for their greater survival success in certain environments (Needham 1991, Gleim et al. 2013).

Although host usage was considered in all models of tick counts, only bobcat presence was an important predictor of *A. americanum* counts, suggesting that host usage did not generally impact tick counts. The failure to identify a significant relationship between host usage and tick counts conflicts with the idea that host abundance significantly affects tick abundance (Paddock and Yabsley 2007, Brunner and Ostfeld 2008, Oorebeek and Kleindorfer 2008). However, a number of manipulative and field-based studies have been inconclusive regarding a correlation between host and tick abundance (Daniels and Fish 1995, Schulze et al. 2001, Jordan and Schulze 2005). In the case of the *A. americanum* model, it could be argued that bobcat presence was acting more as an indicator of other factors impacting *A. americanum* counts rather than a direct relationship between bobcats and *A. americanum* counts. For example, bobcat presence may have been an indicator of small mammal populations which are primary food resources for bobcats and also important hosts for *A. americanum* larvae and nymphs (Childs and Paddock 2003 Godbois et al. 2003). It could also be argued that the correlation between bobcat presence and *A. americanum* was more indicative of habitat types preferred by bobcats (in this case, UBUB) rather than a direct relationship between

bobcats and *A. americanum* counts. However, refuting this idea are studies on bobcat habitat selection in the Southeast which found that they prefer young to mature pine stands, not hardwood areas (Conner et al. 1993, Chamberlain et al. 2003). Ultimately, we suspect that while host abundance may play a minor role in tick dynamics, other factors such as burning and microclimate play larger roles in driving tick dynamics in these particular systems.

In conclusion, operational prescribed burning in pine and mixed pine ecosystems, when performed on a long-term basis, significantly reduces tick populations which supports several previous studies conducted, some of which were performed on smaller plots of land and for shorter periods of time (Jacobson and Hurst 1979, Cully 1999). A number of previous studies showed that although fire kills ticks, they recolonized the area within a few years (Stafford et al. 1998, Davidson et al. 1994, Willis et al. 2012). We suggest that the repeated burnings over long periods of time and the associated harsher microclimates resulting from fire-induced changes in vegetation structure are the primary drivers behind the long-term reductions in tick populations observed. Pathogen testing is still needed to gain a complete understanding of tick-borne disease risk and dynamics in burned areas. Furthermore, studies examining the efficacy of prescribed fires in different management scenarios as well as determining the length of time required to provide overall reductions in tick populations within previously unburned areas is warranted. Nevertheless, long-term prescribed fire used in habitats similar to those investigated in this study appears to be effective at reducing tick populations. These findings illustrate another benefit to utilizing prescribed burning and has important implications for public

health, as it is a time efficient, cost effective method for reducing tick populations and liekly reducing the risk of human tick-borne diseases.

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Table 3.1. Locations and burn information for the 21 sites sampled during this study in Southwest Georgia (unless otherwise noted).

Location	County	Burn Treatment	Burn Interval (years)
JERC, BU 1	Baker	BB	2
JERC, BU 5	Baker	BB	2
JERC, BU 7 East	Baker	BB	2
JERC, BU 7 West	Baker	BB	2
JERC, BU 64	Baker	BB	2
JERC, BU 136	Baker	BB	2
JERC, BU 137	Baker	BB	2
JERC, BU 176	Baker	BB	2
Chickasawhatchee WMA	Calhoun	BUB	2-4
Hannahatchee WMA - site 1	Stewart	BUB	2-4
Hannahatchee WMA - site 2	Stewart	BUB	2-4
River Creek WMA	Thomas	BUB	2-4
Silver Lake WMA	Decatur	BUB	2-4
Flint River WMA	Dooly	UBB	n/a
JERC, BU 107	Baker	UBB	n/a
JERC, BU 140	Baker	UBB	n/a
JERC, BU 15	Baker	UBB	n/a
Lake Seminole WMA - Little Horseshoe Bend Tract	Decatur	UBB	n/a
Montezuma Bluff Natural Area	Macon	UBUB	n/a
Private Property 1	Decatur	UBUB	n/a
Private Property 2	Gladsden, FL	UBUB	n/a

JERC = Joseph W. Jones Ecological Research Center
BU = burn unit
WMA = wildlife management area
BB = burned site, surrounded by burned area
BUB = burned site, surrounded by unburned area
UBB = unburned site, surrounded by burned area
UBUB = unburned site, surrounded by unburned are

Table 3.2. Number of *A. americanum* collected for duration of current study.

Site	Treatment	adult (male/ female)		nymph		larvae (clutches)	
		2010	2011	2010	2011	2010	2011
JERC, BU 1	BB	0	1 (0/1)	0	0	0	0
JERC, BU 5	BB	0	0	0	0	0	0
JERC, BU 7 East	BB	0	0	0	0	0	0
JERC, BU 7 West	BB	0	0	0	0	0	0
JERC, BU 64	BB	0	0	0	0	0	0
JERC, BU 136	BB	0	0	0	0	0	0
JERC, BU 137	BB	0	0	0	0	0	0
JERC, BU 176	BB	0	0	0	0	0	0
Total in BB		**0**	**1**	**0**	**0**	**0**	**0**
Silverlake WMA	BUB	0	0	0	2	0	0
River Creek WMA	BUB	3 (1/2)	2 (1/1)	0	12	0	291 (2)
Hannahatchee WMA , site 2	BUB	1 (0/1)	3 (0/3)	2	2	1362 (1)	0
Hannahatchee WMA, site 1	BUB	0	2 (1/0)*	0	5	0	0
Chickasawhatchee WMA	BUB	0	0	0	0	0	0
Total in BUB		**4**	**7**	**2**	**21**	**1362**	**291**
JERC, BU 15	UBB	0	0	0	0	0	0
JERC, BU 107	UBB	0	0	0	0	0	0
JERC, BU 140	UBB	0	0	0	0	0	0
Horseshoe Bend	UBB	1 (0/1)	1 (0/1)	3	0	0	20 (1)

Flint River WMA	UBB	4 (3/1)	3 (2/1)	21	4	2,354 (2)	
Total in UBB		**5**	**4**	**24**	**4**	**2,354**	**1,177**
Private Property 1	UBUB	42 (22/18)*	63 (35/28)	242	599	16,051 (56)	9,829 (52)
Private Property 2	UBUB	178 (66/112)	80	869	330	10,381 (91)	2,537 (18)
Montezuma Bluff NA	UBUB	52 (26/26)	27 (14/13)	60	52	0	67 (2)**
Total in UBUB		**272**	**170**	**1,171**	**981**	**26,432**	**12,433**
Grand Total by Year		**281 (118/161)***	**182**	**1,197**	**1,006**	**30,148 (91)**	**13,901 (79)**
Grand Total		**463**		**2203**		**44,049 (170)**	

JERC =Jones Ecological Research Center. WMA = Wildlife management area. NA = Natural Area. BB = Burned, surrounded by burned, BUB = Burned, surrounded by unburned, UBB = Unburned, surrounded by unburned, UBUB = Unburned, surrounded by unburned

* sex of remaining ticks was not recorded; **1 group of 40 larvae (not included in the numbers in this table) thought to be a single clutch had both *A. americanum* and *A. maculatum*.

Table 3.3. Number of *A. maculatum* collected for duration of the current study.

Site	Treatment	adult (male/ female)		nymph		larvae (clutches)	
		2010	2011	2010	2011	2010	2011
JERC, BU 1	BB	0	0	0	0	0	0
JERC, BU 5	BB	14 (8/6)	0	1	0	0	0
JERC, BU 7 East	BB	5 (4/1)	0	0	0	0	0
JERC, BU 7 West	BB	6 (5/1)	0	0	0	0	0
JERC, BU 64	BB	0	0	0	0	0	0
JERC, BU 136	BB	2 (1/1)	2 (1/1)	0	0	0	0
JERC, BU 137	BB	2 (1/1)	2 (1/1)	0	0	0	0
JERC, BU 176	BB	1 (1/0)	2 (2/0)	0	0	0	0
Total in BB		**30**	**6**	**1**	**0**	**0**	**0**
Silverlake WMA	BUB	0	0	0	0	0	0
River Creek WMA	BUB	1 (0/1)	1 (0/1)	0	0	0	0
Hannahatchee WMA , site 2	BUB	0	0	0	0	0	0
Hannahatchee WMA, site 1	BUB	23 (11/12)	4 (2/2)	0	1	0	0
Chickasawhatchee WMA	BUB	1 (1/0)	0	0	0	0	0
Total in BUB		**25**	**6**	**0**	**1**	**0**	**0**
JERC, BU 15	UBB	0	0	0	0	0	0
JERC, BU 107	UBB	0	0	0	0	0	0
JERC, BU 140	UBB	0	0	0	0	0	0
Horseshoe Bend	UBB	1 (0/1)	1 (1/0)	0	0	0	0
Flint River WMA	UBB	2 (2/0)	1 (0/1)	0	1	0	0
Total in UBB		**3**	**2**	**0**	**1**	**0**	**0**
Private Property 1	UBUB	0	3 (0/3)	0	0	0	0
Private Property 2	UBUB	0	0	0	0	0	0

Montezuma Bluff NA	UBUB	0	0	0	0	0	**
Total in UBUB		**0**	**3**	**0**	**0**	**0**	**0**
Grand Total by Year		**58 (34/24)**	**16 (7/9)**	**1**	**2**	**0**	**unknown**
Grand Total		**74 (41/33)**		**3**			**unknown**

JERC = J.W. Jones Ecological Research Center. WMA = Wildlife management area. NA = Natural Area. BB = Burned, surrounded by burned, BUB = Burned, surrounded by unburned, UBB = Unburned, surrounded by unburned, UBUB = Unburned, surrounded by unburned

**1 group of 40 larvae (not included in the numbers in this table) thought to be a single clutch had both *A. americanum* and *A. maculatum*

Table 3.4. Number of *I. scapularis* collected for duration of the current study.

Site	Treatment	adult (male/ female)		nymph		larvae (clutches)	
		2010	2011	2010	2011	2010	2011
JERC, BU 1	BB	0	0	0	0	0	0
JERC, BU 5	BB	0	1 (0/1)	0	0	0	0
JERC, BU 7 East	BB	0	0	0	0	0	0
JERC, BU 7 West	BB	0	0	0	0	0	0
JERC, BU 64	BB	1 (0/1)	0	0	0	0	0
JERC, BU 136	BB	1 (0/1)	1 (1/0)	0	0	0	0
JERC, BU 137	BB	0	0	0	0	0	0
JERC, BU 176	BB	0	1 (1/0)	0	0	0	0
Total in BB		**2**	**3**	**0**	**0**	**0**	**0**
Silverlake WMA	BUB	7 (3/4)	5 (3/2)	0	0	0	0
River Creek WMA	BUB	1 (1/0)	8 (4/4)	0	0	0	0
Hannahatchee WMA , site 2	BUB	0	0	0	0	0	0
Hannahatchee WMA, site 1	BUB	0	3 (0/3)	0	0	0	0
Chickasawhatchee WMA	BUB	0	2 (2/0)	0	0	0	0
Total in BUB		**8**	**18**	**0**	**0**	**0**	**0**
JERC, BU 15	UBB	0	0	0	0	0	0
JERC, BU 107	UBB	3 (2/1)	2 (1/1)	0	0	0	0
JERC, BU 140	UBB	3 (3/0)	23 (13/10)	0	0	0	0
Horseshoe Bend	UBB	9 (5/4)	19	0	0	0	0
Flint River WMA	UBB	25 (12/13)	50	1	0	0	0
Total in UBB		**40**	**94**	**1**	**0**	**0**	**0**
Private Property 1	UBUB	26 (1/25)	42 (8/3)	0	0	5 (1)	0
Private Property 2	UBUB	3 (3/0)	8 (6/2)	5	1	0	0
Montezuma Bluff NA	UBUB	18 (3/15)	78	0	0	0	0

Total in UBUB	**47**	**128**	**5**	**1**	**5**	**0**
Grand Total by Year	**97 (33/64)**	**243**	**6**	**1**	**5 (1)**	**0**
Grand Total	**340**		**7**		**5 (1)**	

JERC = J.W. Jones Ecological Research Center. WMA = Wildlife management area. NA = Natural Area. BB = Burned, surrounded by burned, BUB = Burned, surrounded by unburned, UBB = Unburned, surrounded by unburned, UBUB = Unburned, surrounded by unburned.

Table 3.5. Number of *D. variabilis* collected for duration of the current study.

Site	Treatment	adult (male/ female)	
		2010	2011
JERC, BU 1	BB	0	0
JERC, BU 5	BB	0	0
JERC, BU 7 East	BB	0	0
JERC, BU 7 West	BB	0	0
JERC, BU 64	BB	0	0
JERC, BU 136	BB	0	0
JERC, BU 137	BB	0	0
JERC, BU 176	BB	0	0
Total in BB		**0**	**0**
Silverlake WMA	BUB	0	0
River Creek WMA	BUB	7 (5/2)	0
Hannahatchee WMA , site 2	BUB	1 (0/1)	1 (1/0)
Hannahatchee WMA, site 1	BUB	0	0
Chickasawhatchee WMA	BUB	1 (1/0)	1 (1/0)
Total in BUB		**9**	**2**
JERC, BU 15	UBB	0	0
JERC, BU 107	UBB	0	0
JERC, BU 140	UBB	0	0
Horseshoe Bend	UBB	0	0
Flint River WMA	UBB	7 (3/4)	2 (0/2)
Total in UBB		**7**	**2**
Private Property 1	UBUB	3 (1/2)	1 (0/1)
Private Property 2	UBUB	0	0
Montezuma Bluff NA	UBUB	10 (5/5)	4 (4/0)
Total in UBUB		**13**	**5**
Grand Total by Year		**29 (15/14)**	**9 (6/3)**
Grand Total		**38 (21/17)**	

JERC = J.W. Jones Ecological Research Center. WMA = Wildlife management area. NA = Natural Area. BB = Burned, surrounded by burned, BUB = Burned, surrounded by unburned, UBB = Unburned, surrounded by unburned, UBUB = Unburned, surrounded by unburned.

Table 3.6. Generalized estimating equation negative binomial regression model for the prediction of total tick counts at 21 sites in Southwest Georgia in 2010 and 2011.

Variable	Coefficient (SE*)	RR (95% CI)	P
Any Burning			
No	Referent	Referent	
Yes	-1.5 (0.43)	ND	0.001
Year			
2010	Referent	Referent	
2011	0.29 (0.24)	1.3 (0.84, 2.2)	0.22
Season			
Cold (Fall/ Winter)	Referent	Referent	
Hot (Spring/ Summer)	2.4 (0.48)	ND	<0.001
Litter Cover (%)			
0-95	Referent	Referent	
>95	0.81 (0.33)	2.2 (1.2, 4.3)	0.014
Trees per Ha			
0-182	Referent	Referent	
>183	2.3 (0.66)	6.4 (2.6, 35)	0.001
Any Burning X Season			
Yes, Hot	-2.3 (0.59)	ND	<0.001
Constant	-2.0 (0.84)	NA	0.016
ln(Hours flagged)	1	(Effort)	

*SE = Standard error
RR = Relative risk
ND = Not determined, RR is not given because it depends on the interacting variable.
NA = Not applicable

Table 3.7. Generalized estimating equation negative binomial regression model for the prediction of *A. americanum* counts at 21 sites in Southwest Georgia in 2010 and 2011.

Variable	Coefficient (SE*)	RR (95% CI)	P
Any Burning			
No	Referent	Referent	
Yes	-2.5 (1.15)	ND	0.026
Year			
2010	Referent	Referent	
2011	0.07 (0.34)	1.1 (0.55, 2.1)	0.837
Season			
Cold (Fall/ Winter)	Referent	Referent	
Hot (Spring/ Summer)	3.5 (0.45)	ND	<0.001
Bobcats			
Absent	Referent	Referent	
Present	1.2 (0.31)	3.2 (1.7, 5.8)	<0.001
Wind at Ground (mph)	-1.9 (0.54)	0.15 (0.05, 0.44)	<0.001
Any Burning X Season	-2.6 (1.1)	ND	0.019
Constant	1.3 (0.84)	NA	0.717
ln(Hours flagged)	1	(Effort)	

*SE = Standard error
RR = Relative risk
ND = Not determined; RR is not given because it depends on the interacting variable
NA = Not applicable

Table 3.8. Generalized estimating equation negative binomial regression model for the prediction of *I. scapularis* counts at 21 sites in Southwest Georgia in 2010 and 2011.

Variable	Coefficient (SE*)	RR (95% CI)	P
Any Burning			
No	Referent	Referent	
Yes	-1.2 (0.40)	0.28 (0.13, 0.63)	0.002
Year			
2010	Referent	Referent	
2011	1.3 (0.42)	3.8 (1.6, 8.7)	0.002
Season			
Cold (Fall/ Winter)	Referent	Referent	
Hot (Spring/ Summer)	-1.9 (0.47)	0.15 (0.06, 0.37)	<0.001
Trees per Hectare			
0-182	Referent	Referent	
>183	2.9 (0.98)	18 (2.6, 122)	0.003
Precipitation Previous 3 Days (cm)			
0	Referent	Referent	
>0	0.74 (1.1)	2.1 (1.2, 3.8)	0.014
Constant	-3.6 (1.1)	NA	0.001
ln(Hours flagged)	1	(Effort)	

*SE = Standard error
RR = Relative ratio
ND = Not determined; RR is not given because it depends on the interacting variable
NA = Not applicable

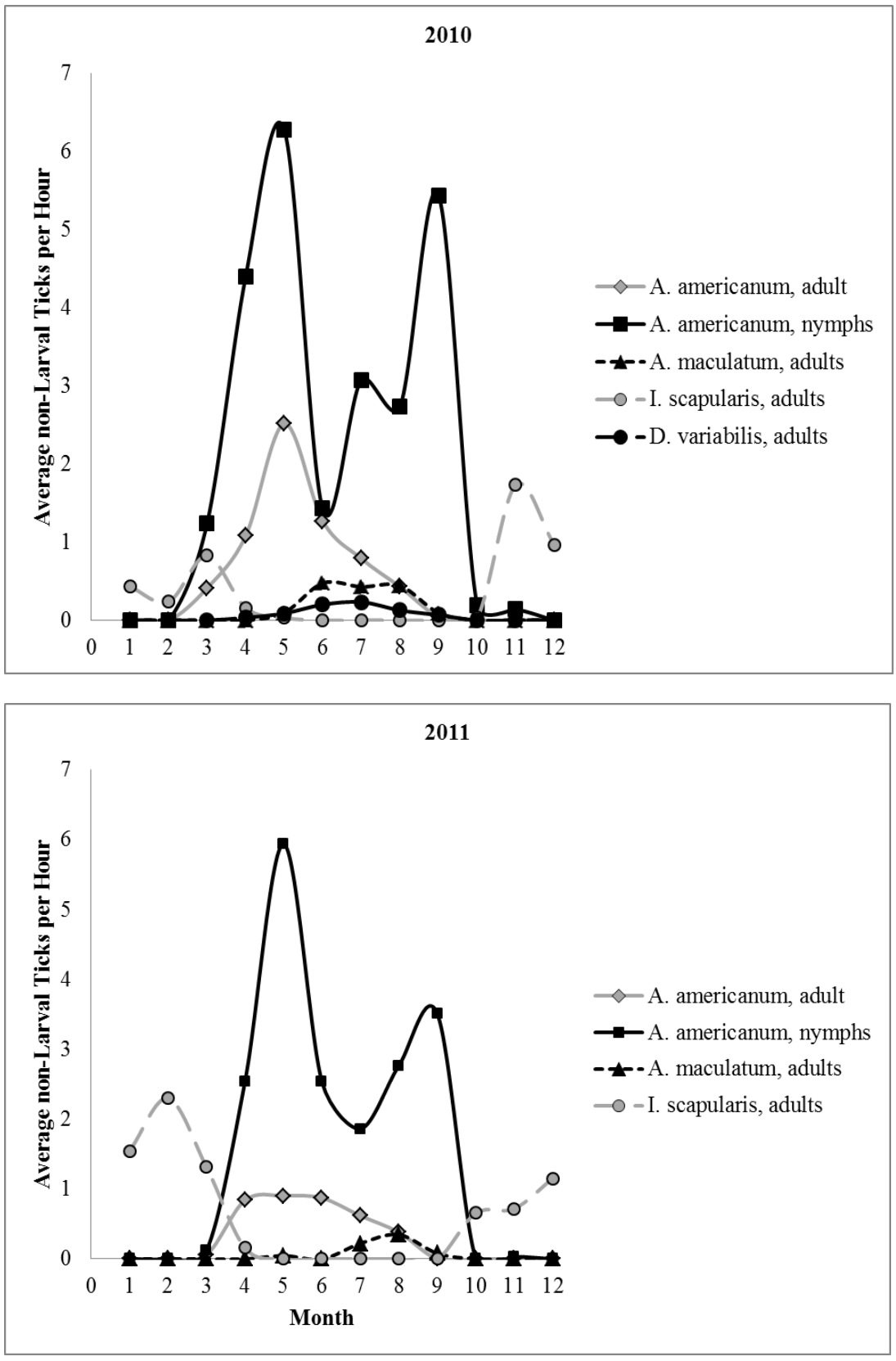

Figure 3.1. Tick seasonality across all study sites. Top: 2010 and Bottom: 2011

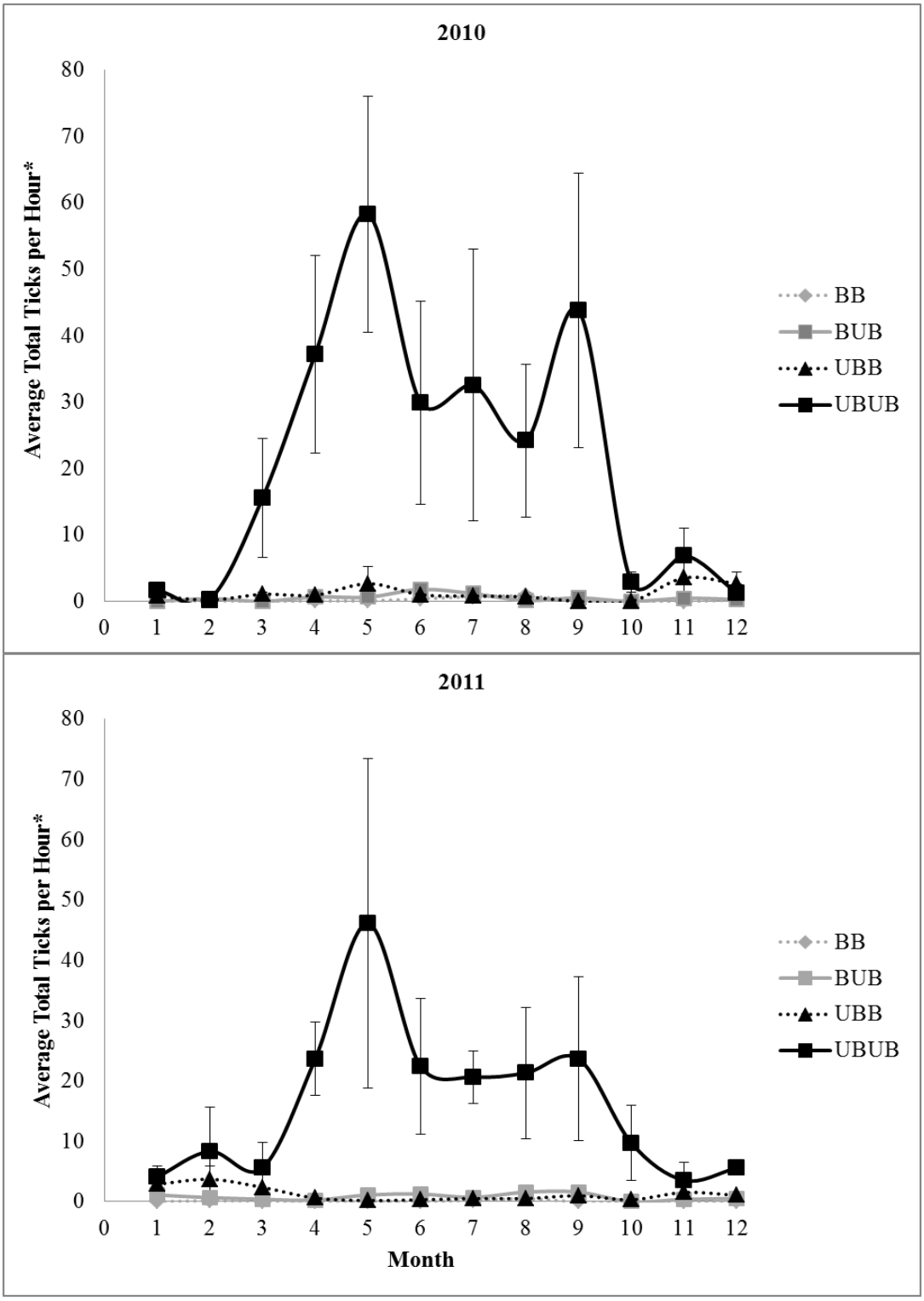

Figure 3.2. Effects of long-term prescribed burning on tick abundance at all study sites throughout Southwest Georgia. Top: 2010 and Bottom: 2011
*One clutch of larvae was counted as a single tick

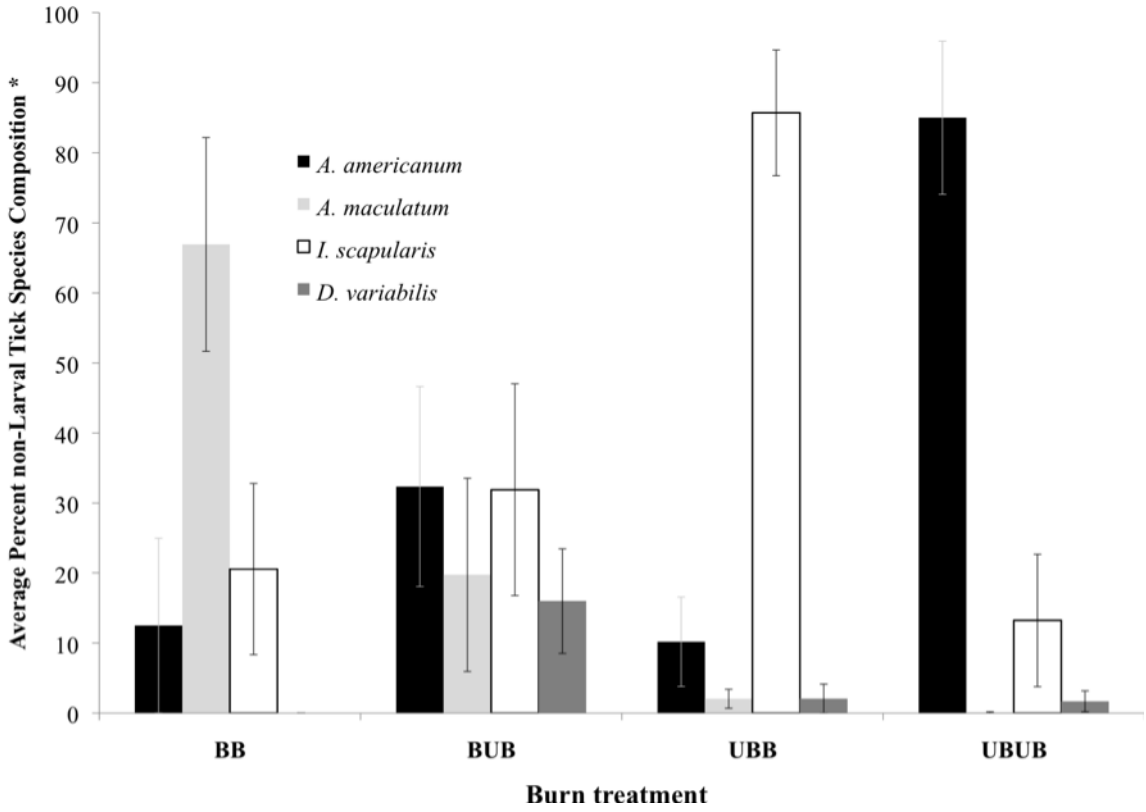

Figure 3.3. Ticks species composition at all study sites in Southwestern Georgia by burn treatment for 2010-2011.

CHAPTER 4

EFFECTS OF VARIABLE BURN REGIMES ON PREVALENCE OF *A. PHAGOCYTOPHILUM*, *EHRLICHIA* SPP., *BORRELIA* SPP., AND *RICKETTSIA* SPP. IN TICKS OF SOUTHWESTERN GEORGIA*

Gleim, E.R., G.E. Zemtsova, M.L. Levin, L.M. Conner, and M.J. Yabsley. To be submitted to *Ticks and Tick-borne Diseases*.

Abstract

Tick-borne disease incidence and emergence has increased dramatically in the past several decades. Understanding tick and tick-borne pathogen dynamics within a region is crucial to preventing and diagnosing tick-borne diseases. Furthermore, identifying methods which may reduce risk of tick-borne diseases are critical. Accordingly, a study was conducted to determine the impacts of long-term prescribed fire on ticks and tick-borne pathogens in Southwestern Georgia. We worked on 21 sites with varying burn regimes (burned surrounded by burned areas [BB], burned surrounded by unburned areas [BUB], unburned surrounded by burned areas [UBB], and unburned surrounded by unburned areas [UBUB]) throughout southwestern Georgia and sampled for ticks via flagging on a monthly basis for two calendar years. In total, 3,546 ticks (3,062 *A. americanum*, 75 *A. maculatum*, 381 *I. scapularis*, two *I. brunneus*, and 26 *D. variabilis*) were tested for 1 or more bacterial pathogen/s including *Rickettsia* spp., *Borrelia spp.*, *Ehrlichia chaffeensis, E. ewingii,* and the Panola Mountain *Ehrlichia* sp. In general, prevalences of each organism by tick species (*Rickettsia* 6.7-66.7%, *Borrelia* 0-2.2%, *E. chaffeensis* 0-1.1%, *E. ewingii* 0-7.2%, and Panola Mountain *Ehrlichia* 0-3.8%) were similar to what has been reported in other regions of Georgia and the southeastern U.S. Interestingly, *A. americanum* from burned habitats had significantly lower minimum infection *Rickettsia* prevalences with *A. americanum* from UBUB sites having a 16.7% prevalence (584/3490) whereas BUB, and UBB sites had 8.7% (19/219) and 4.4% (16/361), respectively. These findings indicate that long-term prescribed fire does not significantly alter infection rates for known pathogenic bacteria (e.g., *Ehrlichia* spp.), but because numbers of *A. americanum* are greatly reduced in burned habitats, the overall

risk to people should be reduced. However, our data indicate that there may be an increased risk of *R. parkeri* infection to people in burned areas due to its vector, *A. maculatum* dominating in burned areas.

Introduction

There are a number of ticks of public health significance in Georgia including *Amblyomma americanum*, *Dermacentor variabilis*, *Ixodes scapularis*, and *A. maculatum* (Georgia Department of Human Resources 2008). All of these ticks are capable of transmitting one or more tick-borne pathogens. For example, *A. americanum* is the main vector of *Ehrlichia chaffeensis (*human monocytic ehrlichiosis [HME]), *E. ewingii* (*Ehrlichia ewingii* ehrlichiosis), and Panola Mountain *Ehrlichia* (Panola Mountain ehrlichiosis). Ehrlichiosis is generally associated with a mild to moderate febrile illness but, in rare instances, HME can be fatal (Shah et al. 2012). *A. americanum* is also the vector of the causative agent of Southern Tick-Associated Rash Illness (STARI). Although the etiologic agent of STARI has not yet been confirmed, *Borrelia lonestari* and *Rickettsia amblyommii* have been considered as potential causative agents (James et al. 2001, Billeter et al. 2007)

Other tick-borne pathogens of interest in Georgia include *Rickettsia rickettsii* (Rock Mountain spotted fever [RMSF]) transmitted by *D. variabilis*, *R. parkeri* (American boutonneuse fever) transmitted by *A. maculatum*, and *B. burgdorferi* (Lyme disease) and *A. phagocytophilum* (human granulocytic anaplasmosis [HGA]) both transmitted by *I. scapularis*. In Georgia, many of these tick-borne diseases are reportable but are probably underreported due to cases being empirically treated without diagnostic

follow-up or patients not seeking medical attention. Regardless, in 2008, the Georgia Department of Public Health (GDPH) reported that there were 10 confirmed and 66 probable cases of RMSF, 21 confirmed and 8 probable cases of Lyme disease, 5 confirmed and 13 probable cases of HME, and 1 probable case of HGA (Garrison 2009). Interestingly, there was a substantial increase in the number of Lyme cases reported in 2008 compared with previous years. While this was in part due to changes in case definitions used by GDPH, it was noted that even when data were analyzed using the previous case definition, there were still approximately 30% more cases compared with previous years (Garrison 2009).

In fact, across the United States, the incidence of tick-borne diseases has increased and several new diseases have emerged (Maeda et al. 1987, Parola et al. 2005, Goodin et al. 2006, Apperson et al. 2008). This increase of tick-borne disease has been linked to land management practices, improved diagnostics, and increased numbers of high risk individuals (i.e., immunosuppressed and elderly) (Allan et al. 2003, Patz et al. 2005, Goodin et al. 2006, Apperson et al. 2008). Recently, Gleim et al. (Chapter 3) found that long-term prescribed fire, a common land management practice, significantly reduced and altered tick species composition. While it is thought that changes in land management and usage have contributed to increases in tick populations, it is less clear how differences in land use and management affect pathogen prevalence (LoGuidice et al. 2003, Manangan et al. 2007, Paddock and Yabsley 2007, Karelis et al. 2012).

The current study was conducted to determine the prevalence of tick-borne pathogens relevant to human public health in southwestern Georgia, a region of the state in which little is known regarding tick and tick-borne pathogen dynamics. To follow-up

on the finding that long-term prescribed fire significantly reduced tick abundance (Gleim et al. Chapter 3), the effects of long-term prescribed burning on pathogen prevalence was evaluated to investigate how this particular land management practice may affect pathogen dynamics and ultimately the risk of human infection.

Materials and Methods

Study Area

This study was conducted in southwestern Georgia which is dominated by pine and mixed pine forests, as well as agriculture. Prescribed burning is commonly used throughout the region to maintain various pine ecosystems including, longleaf pine ecosystems. Twenty-one sites were selected based on the presence or absence of long-term prescribed burning, with burn-present sites having had at least 10 years of regular prescribed burns or lack thereof (Table 4.1). To account for land cover and management both within the site and immediately surrounding the site, each site was further categorized as being 1) burned surrounded by burned (BB), 2) burned surrounded by unburned (BUB), 3) unburned surrounded by burned (UBB) and 4) unburned surrounded by unburned (UBUB) (i.e., a control).

Tick Collections

Each site was sampled for ticks on a monthly basis for two calendar years (January 2010-December 2011) using 1m x 1m flags made of flannel cloth (Gleim et al. unpublished). Flags were checked every 10 minutes and all nymphs and adults were collected and preserved in 70% ethanol. All larvae were removed en masse with masking tape with each clutch being kept separate.

Tick Identifications

All adult and nymphal *Amblyomma* spp. and adult *Dermacentor variabilis* (Acari: Ixodidae, Say), and *Ixodes* were morphologically identified utilizing a microscope and key (Kierans and Litwak, 1989; Kierans and Durden 1998). All larvae, *Ixodes* spp. nymphs, and a subset of adult *Ixodes* spp. were confirmed through amplification and sequence analysis. For adults and nymphs, all ethanol was allowed to evaporate off and adults were bisected with a scalpel prior to extraction. DNA was extracted from ticks using a DNeasy blood and tissue kit (QIAGEN, Valencia, CA) utilizing the manufacturer's protocol. For *Amblyomma* sp. ticks collected in 2010, a multiplex real-time polymerase chain reaction (qPCR) was used to differentiate between *A. maculatum* and *A. americanum* larvae targeting the ITS2 gene using Aame_ITS2F/Aame_ITS2R and Amac_ITS2F/AmacITS2R (Zemtsova unpublished). For *Amblyomma* ticks collected in 2011 and all *Ixodes* sp., a PCR targeting the 16S rDNA gene using the 16S-1 and 16S+2 primers was conducted as described in Black and Piesman (1994). DNA extraction and PCR reactions were performed in hoods dedicated strictly for their respective tasks and waters were included as negative controls to detect contamination. All PCR products from the 16S protocol were purified using a QIAquick gel extraction kit (QIAGEN) and bi-directionally sequenced at the Georgia Genomics Facility (Athens, GA).

Pathogen Testing

All adult ticks were individually extracted and tested. Nymphs of the same species and from the same site and date were extracted in pools of five. For larvae, a maximum of 100 larvae of the same species and from the same site and date were extracted in pools of 20 with each pool being from a different clutch if possible. Because

the same sites were sampled in 2011, only five pools of *A. americanum* nymphs from each site were tested for pathogens in 2011. All DNA was stored at -20°C until PCR testing.

In 2010, all *Amblyomma* spp. were screened for *Rickettsia* spp., *E. chaffeensis* and *E. ewingii* using a multiplex quantitative polymerase chain reaction (qPCR) targeting the 17kDa gene of *Rickettsia* sp. and the 16S rRNA gene for both *Ehrlichia* species using primers Ech16S-17/ Ech16S-99, and probe Ech16S-FAM, Ech16S-17/ Ech16S-99, and probe EEW16S HEX, and R17K135F/R17K249R, and probe R17KBC (Killmaster unpublished). To identify *Rickettsia* spp., all samples positive from the multiplex assay were analyzed using a restriction fragment-length polymorphism (RFLP) assay targeting the rOmpA gene using primers RR190.70 and RR190.701R (Roux et al. 1996) followed by the restriction enzymes *RsaI* and *PstI* (Eremeeva et al. 1994).

In 2011, *Amblyomma* sp. and *Ixodes* sp. were tested for *Rickettsia* sp. utilizing a nested PCR targeting the 17kDa gene using 17kD5/17kD3 primers for the primary reaction and 17kD1/17kD2 primers for the secondary reaction (Labruna et al. 2004). Approximately half of the *Rickettsia* sp. positive samples from 2011 were purified using a QIAquick gel extraction kit and sequenced at the Georgia Genomics Facility (Athens, GA). All *A. americanum* from 2011 were tested for *E. chaffeensis* using nested PCR targeting the 16S rRNA gene using primers ECC/ECB for the primary reaction and HE1/HE3 for the secondary reaction (Dawson et al. 1994). Similarly, *E. ewingii* was tested for using a nested PCR targeting the 16S rRNA gene using primers ECC/ECB for the primary reaction and HE3/EE72 for the secondary reaction (Dawson et al. 1994, Dawson et al. 1996).

All *A. americanum* and *Ixodes* spp. were tested for *Borrelia* sp. using a nested PCR protocol targeting the *flab* gene using FLALL/FLARL primers for the primary reactions and FLALS/FLARS primers for the secondary reactions (Barbour et al. 1996). All *Ixodes* spp. were tested for *Anaplasma* spp. using a PCR assay targeting the *msp2* gene using msp2-3f/msp2-3r primers (Massung and Slater 2003). All positive samples from 2010 were identified by bi-directionally sequencing at the Centers for Disease Control and Prevention (Atlanta, GA).

All DNA extraction, primary, and secondary reactions were conducted in separate areas of the laboratory designated for that purpose. A negative control (i.e., water) was included with each batch of extractions and PCR reactions. Appropriate positive controls were included in all batches of PCR.

Statistics

An analysis of variance (ANOVA) (STATA 12.1 2011) was used to determine the effects of burn treatment on minimum infection prevalence of each tick species.

Results

In total 3,546 ticks were tested for 1 or more pathogen/s (3,062 *A. americanum*, 75 *A. maculatum*, 381 *I. scapularis*, two *I. brunneus*, and 26 *D. variabilis*). Burn treatments were found to have a significant effect on the minimum infection prevalence of *Rickettsia* spp. in *A. americanum* ($F = 2.69$; $df = 3, 887$; $p = 0.0455$) with BUB, UBB, and UBUB having 8.7% (19/219), 4.4% (16/361), and 16.7% (584/3490) prevalence respectively (Table 4.2). The BB study site only had a single *A. americanum*, which was

positive. Burn treatment was not found to have a significant effect on any other tick species and/or pathogen.

In *A. maculatum* adults, 20% (n = 74) were positive for *Rickettsia* spp.; however, only two of the 15 positives could be identified to species, of which one was positive for *R. parkeri* (1.4%) (Table 4.2). In *A. americanum*, overall *Rickettsia* spp. prevalence ranged between 3.5 - 80% based on the year and lifestage. Among the *Rickettsia* identified from *A. americanum* (n = 444) (Table 4.2), 95% were identified as *R. amblyommii*. In *I. scapularis* adults, 48% were positive for *Rickettsia* sp. with the most commonly identified being *Rickettsia* sp. TR-39. Other endosymbionts identified in *I. scapularis* included *R. cooleyi*, *R. monacensis*, *R. amblyommii*, and *Rickettsia* sp. TX140 (Table 4.2). In *D. variabilis* adults, 42% were positive for *Rickettsia* spp. and while a number of endosymbionts were detected including *R. rhipicephali*, *R. amblyommii*, and *Rickettsia* sp. TR-39, importantly, no *R. rickettsii* was identified.

Data of *Ehrlichia* spp. testing are shown in Table 4.3. In 2010, only *A. americanum* were tested for *Ehrlichia* spp., whereas in 2011, both *A. americanum* and *D. variabilis* were tested. In *A. americanum* adults from 2011 and 2012, prevalence rates of 0.6%, 4.4%, and 1.0% (n = 473) were detected for *E. chaffeensis*, *E. ewingii*, and Panola Mountain *Ehrlichia* sp., respectively. Among *A. americanum* nymphs, minimum infection prevalences of 0.2% and 0.3% (n = 1189) were detected for *E. chaffeensis* in 2010 and 2011 respectively and a 0.3% prevalence of Panola Mountain *Ehrlichia* sp. in 2010. In 2010, a single *D. variabilis* (4%, n=26) was positive for *E. ewingii*. With the exception of a single *E. ewingii* positive tick from a BUB site, all other *Ehrlichia* spp.

positive ticks originated from UBUB sites. All *A. maculatum* (n = 58) and *A. americanum* larvae (n = 1400) were negative for all three *Ehrlichia* spp.

Borrelia infections were rare and none were detected in *A. maculatum*, *I. scapularis*, or *I. brunneus* (Table 4.5). *B. lonestari* was detected in *A. americanum* adults (n = 427), nymphs (n = 1189), and larvae (n = 1400) at 1.5%, 0.9%, and 0.1% prevalences, respectively. Similarly, *A. phagocytophilium* was rare with only 1.1% of adult *I. scapularis* being positive. None of the I. *scapularis* nymphs or *I. brunneus* were positive.

Discussion

To our knowledge, this was the first large-scale, tick-borne pathogen survey performed in southwestern Georgia, thus providing valuable insight into tick-borne pathogen dynamics in the region. Pathogen prevalences were similar to what has been reported in other parts of Georgia and neighboring states (Whitlock at al. 2000, Cohen et al. 2010). For example Clark (2004) reported 0-4.8% prevalence of *Borrelia lonestari* by site in northern Florida, while Mixson et al. (2006) reported an average prevalence of 2.1% and 3.4% respectively for *Ehrlichia chaffeensis* and *E. ewingii* from sites around Georgia.

Of note were the relatively high prevalences and high diversity of *Rickettsia* spp. endosymbionts in *D. variabilis* (42%) and *I. scapularis* (48%) in contrast to the complete absence of known pathogenic bacteria (i.e., *Borrelia burgdorferi* and *B. miyamoti* in *I. scapularis* and *R. rickettsii* in *D. variabilis).* The absence of *B. burgdorferi* and low prevalence of *A. phagocytophilum* in *I. scapularis* seems to agree with other studies of

these pathogens in the southeastern United States which have found that prevalence rates are significantly lower compared to prevalence rates in the northeastern and midwestern U.S (Yabsley et al. 2009, Rosen et al. 2012, Stromdahl and Hickling 2012). For example, Fang et al. (2002) tested *I. scapularis* for *A. phagocytophilum* from 15 sites throughout South Carolina, Georgia, and Florida and found prevalences ranging between 0-4.1% with the exception of Jekyll Island, Georgia which had a prevalence of 20%.

The cause of this disparity in pathogen prevalence between the north- and southeastern U.S. is not entirely understood; however, it is suspected that differences in host ecology may play a role. Indeed, both pathogens share rodent reservoirs (Donahue et al. 1987, Anderson 2006) and while studies investigating tick infestations on rodents in the Northeast often find nearly 100% infestation prevalences (Stafford et al. 1999), a study performed in northwestern Florida (32 km from River Creek WMA in this study) only found on average a 3% infestation rate (Durden et al. 2000). Oliver et al. (2003) found slightly higher infestation rates (0-24% by site) when collecting ticks from *Peromyscus gossypinus* (the only rodent species collected that was infested) in southern Georgia and northern Florida and similar infestation rates of *I. minor* and *I. affinis* (suspected to play an important role in *B. burgodorferi* maintenance in the southeastern U.S. [Oliver 1996, Clark et al. 2001]), although it is unclear how many individuals were co-infested. Regardless of whether the lower infestation rates observed in this region are due to lower tick densities or differences in host preferences as compared to the Northeast, it is likely that this would impact maintenance and transmission of *B. burgdorferi* and possibly explain the absence of it in our study.

In the case of *D. variabilis*, the low number of ticks tested may have resulted in an inaccurate portrayal of *Rickettsia* spp. dynamics and specifically the likelihood of us detecting *R. rickettsii*. However, previous studies have typically found much lower Spotted Fever Group (SFG) Rickettsia prevalences than what was detected in this study (Magnarelli et al. 1981, Stromdhal et al. 2001, Schreifer and Azad 1994). For example, in Maryland prevalences of 3.8% (Ammerman et al. 2004) for SFG Rickettsia were documented while a study in Ohio documented 0.2% prevalence (Pretzman et al. 1990). Moving farther southward, Loving et al. (1978) documented a 2.4-3.9% prevalence of SFG Rickettsia over a 3-year period in South Carolina. Interestingly, it has been found that the non-pathogenic SFG Rickettsia, *R. peacockii* inhibits transovarial transmission of *R. rickettsii*, thus limiting its distribution in some areas (Burgdorfer et al. 1981, Macaluso and Azad 2005). Indeed, Dergousoff et al. (2009) found a 76% prevalence of *R. peacokii* in *D. variabilis* and *D. andersoni* in Canada, while finding no *R. rickettsii*. It is difficult to draw any conclusions in the current study due to the low sample size of *D. variabilis*; however, the high prevalence and diversity of non-pathogenic SFG *Rickettsia* spp. observed in *D. variabilis* in this study may be playing a role in driving *R. rickettsii* dynamics within the region.

Rickettsia parkeri is of significant public health importance as it is the causative agent of American boutonneuse fever, with the first human case being identified in 2004 (Paddock et al. 2004). Since then, a number of other cases have been identified (Raoult and Paddock 2005, Finley et al. 2006, Whitman et al. 2007), and the need to learn more about this pathogen and its somewhat uncommon vector has become apparent. In this study, *A.maculatum* was found to have an overall prevalence of *Rickettsia* spp. of 20%

with *R. parkeri* having a minimum prevalence of 1.3%. Unfortunately, a large number of the *Rickettsia* spp. could not be identified to species. However, it is likely that some of these *Rickettsia* spp. were *R. parkeri* given the fact that it is typically found in relatively high prevalences as compared to other pathogenic bacteria in other tick species (e.g. *E. chaffeensis, E. ewingii, R.* rickettsii, etc). For example, Sumner et al. (2007) tested *A. maculatum* from throughout the eastern United States and found on average a 10.5% prevalence (n = 255). Some studies have reported much higher prevalences; indeed, Fornadel et al (2010) found a prevalence of 41% (n = 507) in *A. maculatum* collected in Fairfax County, VA.

Few studies have evaluated the impacts of long-term prescribed fire on actual tick-borne pathogen prevalence. Interestingly, Gleim et al. (Chapter 3) found that burning significantly decreased *A. americanum, I. scapularis,* and overall tick abundance at the current study sites. Furthermore, differences in tick composition among the sites were observed with *A. maculatum* dominating in BB sites, *A. americanum* dominating at UBUB sites, *I. scapularis* dominating at UBB sites and an even distribution of species at BUB sites. These findings in conjunction with our current pathogen testing have allowed us to gain direct insight into the impacts that burning may have on disease risk via calculating the number of infected ticks encountered per hour within each treatment. Specifically, when using the overall prevalence of pathogenic bacteria (i.e. *E. chaffeensis, E. ewingii*, Panola Mountain *Ehrlichia*, *A. phagocytophilum,* and *R. parkeri*) in conjunction with the peak average number of non-larval ticks collected as determined in Gleim et al. (unpublished), it was found that the number of infected ticks per hour was 0.02 (overall pathogenic bacteria prevalence, peak average number of non-larval ticks per

hour (2.4%, 0.79), 0.02 (1.1%, 1.8), 0.02 (0.5%, 3.72), and 0.70 (1.2%, 58.3) for BB, BUB, UBB, and UBUB respectively. Thus, while pathogen prevalence is not significantly different in burned versus unburned sites, the reduction in ticks, despite the shift in tick species composition, indicates that there are 35 times fewer infected ticks in sites with long-term prescribed burning.

Our finding that UBUB sites had higher prevalences of *Rickettsia* spp. in *A. americanum* than in burned sites seems to indicate that burning not only reduces the density of pathogen-infected ticks but also alters or interrupts endosymbiotic *Rickettsia* spp. maintenance. Endosymbiotic *Rickettsia* spp. are typically not pathogenic, so this finding does not have any implications for public health. However, there have been some recent reports suggesting the possibility that *R. amblyommii*, a common endosymbiont in *A. americanum* detected in this study, could be pathogenic in some cases (Billeter et al. 2007, Gleim et al. unpublished). Because *Rickettsia* spp. are primarily maintained through transovarial transmission (as opposed to wildlife hosts) (Socolovschi et al. 2009), it is unlikely that differences in host composition between burned and unburned sites are the cause of this decreased prevalence. It is suspected that the actual decrease in prevalence at burned sites may be due to the significant, long-term reduction of tick populations observed at burned study sites (Gleim et al. Chapter 3). Such long-term reductions, particularly of *A. americanum* which typically have exceptionally high prevalences of *Rickettisa* spp. (Stromdahl et al. 2008, Castellaw et al. 2010, Jiang et al. 2010), could lead to reduced transmission and overall lower ubiquity of this bacteria.

Conclusion

This study provided valuable information on tick-borne pathogen dynamics in southwestern Georgia, a relatively unstudied region. Furthermore, our finding that long-term prescribed burning reduces the density of ticks infected with pathogenic bacteria has significant implications for public health as this would result in a decreased risk of disease. Further investigation into this management practice and its efficacy for use in areas with high tick-borne disease risk may be warranted. While impacts as dramatic as those observed within the current study sites may take multiple burn cycles over a number of years, it is a relatively time- and cost-effective method for significantly reducing tick-borne disease risk.

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Table 4.1. Locations and burn information for the 21 sites sampled during this study in Southwest Georgia (unless otherwise noted).

Location	County	Burn Treatment	Burn Interval (years)
JERC, BU 1	Baker	BB	2
JERC, BU 5	Baker	BB	2
JERC, BU 7 East	Baker	BB	2
JERC, BU 7 West	Baker	BB	2
JERC, BU 64	Baker	BB	2
JERC, BU 136	Baker	BB	2
JERC, BU 137	Baker	BB	2
JERC, BU 176	Baker	BB	2
Chickasawhatchee WMA	Calhoun	BUB	2-4
Hannahatchee WMA - site 1	Stewart	BUB	2-4
Hannahatchee WMA - site 2	Stewart	BUB	2-4
River Creek WMA	Thomas	BUB	2-4
Silver Lake WMA	Decatur	BUB	2-4
Flint River WMA	Dooly	UBB	n/a
JERC, BU 107	Baker	UBB	n/a
JERC, BU 140	Baker	UBB	n/a
JERC, BU 15	Baker	UBB	n/a
Lake Seminole WMA - Little Horseshoe Bend Tract	Decatur	UBB	n/a
Montezuma Bluff Natural Area	Macon	UBUB	n/a
Private Property 1	Decatur	UBUB	n/a
Private Property 2	Gladsden, FL	UBUB	n/a

JERC = Joseph W. Jones Ecological Research Center
BU = burn unit
WMA = wildlife management area
BB = burned site, surrounded by burned area
BUB = burned site, surrounded by unburned area
UBB = unburned site, surrounded by burned area
UBUB = unburned site, surrounded by unburned are

Table 4.2: Results of *Rickettsia* spp. testing of ticks collected at sites in Southwestern Georgia in 2010 and 2011.

	2010					2011				
	Total *	adult	nymph**	larvae	Organisms Detected Ŧ	**Total**	adult	nymph	larvae	Organisms DetectedŦ
A. americanum										
BB	**-**	-	-	-		**1/1 (100)**	1/1 (100)	-	-	1 *R. amblyommii* (96%, 99%, GQ302891)
BUB	**7/96 (7.3)**	5/9 (55.6)	0/4 (0)	2/83 (2.4)	*5 R. amblyommii* 2 *Rickettsia sp.*	**12/123 (9.8)**	4/6 (66.7)	4/17 (23.5)	4/100 (4.0)	9 *R. amblyommii* *1 Rickettsia* sp. (95%, 97%, EU826507) 1 *Rickettsia* sp. (96%, 99%, GQ302897) 1 *Rickettsia* sp.
UBB	**11/233 (4.7)**	4/20 (20.0)	2/12 (16.7)	5/201 (2.5)	10 *R. amblyommii* 1 *Rickettsia* sp.	**5/128 (3.9)**	4/4 (100)	0/4 (0)	1/120 (0.8)	3 *R. amblyommii* 2 *Rickettsia* sp.
UBUB	**379/1911 (19.8)**	145/274	190/1065 (17.8)	44/1,116 (3.9)		**205/1,579 (13.0)**	135/169 (79.9)	17/82 (20.7)	53/1328 (4.0)	87 *R. amblyommii*

		(52.9)			310 *R. amblyommii* 69 *Rickettsia* sp.				5 *R. amblyommii* (70-100%, 95-98%, KC003473) 8 *R. amblyommii* (74-100%, 93-99%, CP003334) 1 *R. amblyommii* (83%, 74%, EU828788) 1 *Rickettsia* sp. (95%, 98%, DQ092218) 1 *Rickettsia* sp. (98%, 99%, GQ302897) 102 *Rickettsia* sp.
A. maculatum									
BB	**8/32 (25.0)**	8/31 (25.8)	0/1 (0)	-	1 *R. amblyommii* 1 *R.*	**0/6 (0)**	0/6 (0)	-	-

					parkeri 6 *Rickettsia* sp.				
BUB	**4/24 (16.7)**	4/24 (16.7)	-	-	*4 Rickettsia* sp.	**0/4 (0)**	0/4 (0)	-	-
UBB	**2/3 (66.7)**	2/3 (66.7)	-	-	*2 Rickettsia* sp.	**0/2 (0)**	0/2 (0)	-	-
UBUB	-	-	-	-		**1/3 (33.3)**	1/3 (33.3)	-	-	1 *R. amblyommii*
I.scapularis									
BB						**0/2 (0)**	0/2 (0)	-	-	
BUB						**9/17 (52.9)**	9/17 (52.9)	-	-	1 *R. cooleyi* 3 *Rickettsia* sp. TR-39 2 *Rickettsia* sp. TR-39 (96%, 96-99%, DQ480762) 1 *Rickettsia* sp. (95%, 97%, KC003474) 2 *Rickettsia* sp.
UBB						**40/102 (39.2)**	40/102 (39.2)	-	-	1 *R. amblyommii*

UBUB	**73/132 (55.3)**	73/131 (55.7)	0/1 (0)	-	3 *R. monacensis* 17 *Rickettsia* sp. TR-39 2 *Rickettsia* sp. (89-97%, 96-98%, KC003474) 1 *Rickettsia* sp. (99%, 99%, JN190456) 13 *Rickettsia* sp. 2 *R. cooleyi* 1 *R. monacensis* 15 *Rickettsia* sp. TR-39 5 *Rickettsia* sp. TR-39 (96-99%, 92-99%, DQ480762) 4 *Rickettsia* sp. TR-39 (93-96%,

									99-100%, EF689739) 4 *Rickettsia* sp. TX140 (93-96%, 99-100%, EF689739) 1 *Rickettsia* sp. (67%, 84%, KC003474) 1 *Rickettsia* sp. (98%, 97%, EU283838) 40 *Rickettsia* sp.
D. variabilis									
BB	**0/1 (0)**	0/1 (0)	-	-		-	-	-	
BUB	**0/6 (0)**	0/5 (0)	-	-	**2/2 (100)**	2/2 (100)	-	-	1 *Rickettsia* sp. TR-39 (92%, 98%, DQ480762) 1 *R. amblyommii* (87%, 79%, GQ302891)

UBB	**1/7 (14.3)**	1/7 (14.3)	-	-	1 *R. amblyommii*	**1/2 (50.0)**	1/2 (50)	-	-	1 *R. rhipicephali*
UBUB	**8/13 (61.5)**	8/13 (61.5)	-	-	8 *Rickettsia* sp.	**3/5 (60.0)**	3/5 (60.0)	-	-	3 *R. rhipicephali*

*Number of infected ticks (or pools when applicable) over total ticks tested (percent positive).
**For nymphs and larvae, indicates minimum infection prevalence.
Ɨ For 2010 & 2011, Rickettsia sp. indicates a positive sample that was not possible to sequence or was not sent for sequencing.
Ⱨ For 2011 only, all samples were sequenced having a minimum of 97% sequence coverage and identity unless otherwise noted.

Table 4.3. Results of *Ehrlichia* spp. testing of ticks collected in Southwestern Georgia.

		2010						2011		
		A. maculatum		*A. americanum*			*D. variabilis*	*A. americanum*		
	Pathogen	adult	nymph	adult	nymph	larvae	adult	adult	nymph	**Total**
BB		0/30*	0/1	-	-	-	-	0/1	-	0/32
BUB		0/24	-	0/10	0/4	0/83	0/6	0/6	0/17	0/150
UBB	*E. chaffensis*	0/3	-	0/9	0/12	0/201	0/7	0/4	0/4	0/240
UBUB		-	-	1/274 (0.4)	2/1070 (0.2)**	0/1116	0/13	2/169 (1.2)	0/82	5/2724 (0.2)**
Total		**0/57**	**0/1**	**1/293 (0.3)**	**2/1086 (0.2)****	**0/1400**	**0/26**	**2/180 (1.1)**	**0/103**	**5/3146 (0.2)****
BB		0/30*	0/1	-	-	-	-	0/1	-	0/32
BUB		0/24	-	0/10	0/4	0/83	1/6 (16.7)	0/6	0/17	1/150 (0.7)
UBB	*E. ewingii*	0/3	-	0/9	0/12	0/201	0/7	0/4	0/4	0/240
UBUB		-	-	8/278 (2.9)	0/1070	0/1116	0/13	13/169 (7.7)	0/82	21/2724 (0.8)
Total		**0/57**	**0/1**	**8/293 (2.7)**	**0/1086**	**0/1400**	**1/26 (3.8)**	**13/180 (7.2)**	**0/103**	**22/3146 (0.7)**

BB		0/30*	0/1	-	-	-	0	-	-	0/32
BUB		0/24	-	0/10	0/4	0/83	0/6	-	-	0/150
UBB	Panola Mountain *Ehrlichia*	0/3	-	0/9	0/12	0/201	0/7	-	-	0/240
UBUB		-	-	3/274 (1.1)	3/1070 (0.3)**	0/1116	1/13 (7.7)	-	-	7/2724 (0.2)**
Total		**0/57**	**0/1**	**3/293 (1.0)**	**3/1086 (0.3)****	**0/1400**	**1/26 (3.8)**	-	-	**7/3146 (0.2)****

* number of infected ticks (or pools when applicable) over total ticks tested (percent positive).
**minimum infection prevalence.

Table 4.4. Results of *A. phagocytophilum* testing of ticks from Southwestern Georgia.

	2010		2011			
	I. scapularis		*I. scapularis*		*I. brunneus*	
	adult	nymph	adult	nymph	nymph	**Total**
BB	0/3 (0)*	-	0/2 (0)	-	-	**0/5 (0)**
BUB	1/12 (8.3)	-	0/18 (0)	-	-	**1/30 (3.3)**
UBB	2/43 (4.6)	0/1 (0)	0/98 (0)	-	0/2 (0)	**2/144 (1.4)**
UBUB	0/62 (0)	0/11 (0)	1/130 (7.7)	0/1 (0)	-	**1/204 (0.5)**
Total	**3/120 (2.5)**	**0/12 (0)**	**1/248 (0.4)**	**0/1 (0)**	**0/2 (0)**	**4/383 (1.0)**

* number of infected ticks over total ticks tested (percent positive)

Table 4.5. Results of *Borrelia* spp. testing of ticks from southwestern Georgia. Note that all positives were identified as *B. lonestari*.

	2010					2011		
	A. maculatum		*A. americanum*			*A. americanum*		
	adult	nymph	adult	nymph**	larvae**	adult	nymph	**Total**
BB	0/30 (0)*	0/1 (0)	-	-	-	0/1 (0)	-	**0/32 (0)**
BUB	0/24 (0)	-	1/10 (10.0)	0/4 (0)	1/83 (0.1)	0/6 (0)	0/17 (0)	**2/144 (1.4)**
UBB	0/3 (0)	-	0/9 (0)	0/15 (0)	5/201 (0.2)	0/4 (0)	0/4 (0)	**5/240 (2.1)**
UBUB	-	-	2/274 (0.7)	11/1070 (0.1)	14/1116 (0.1)	4/168 (2.4)	0/82 (0)	**31/2723 (1.4)**
Total	**0/57 (0)**	**0/1 (0)**	**3/293 (0.1)**	**11/1086 (0.1)**	**20/1400 (0.1)**	**4/179 (2.2)**	**0/103 (0)**	**38/3101 (1.4)**

* number of infected ticks (or pools when applicable) over total ticks tested (percent positive)

**minimum infection prevalence

CHAPTER 5

THE EFFECTS OF *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE) AND BURNED HABITAT ON THE SURVIVAL OF *AMBLYOMMA AMERICANUM* (ACARI: IXODIDAE) AND *AMBLYOMMA MACULATUM* (ACARI: IXODIDAE)*

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Abstract

Identifying ways in which humans can reduce tick populations is important for preventing the spread and emergence of diseases. During a recent study on effects of long-term prescribed burning on ticks, differences in species composition were observed with lone star ticks, *Amblyomma americanum* (L.), preferring unburned habitats and Gulf Coast ticks, *Amblyomma maculatum* (Koch), preferring burned habitats. Interestingly, the red imported fire ant (RIFA), *Solenopsis invicta* Buren, is found predominantly in disturbed habitats, such as burned habitats, and studies have reported that RIFA prey on lone star ticks. To better understand drivers of tick population differences in burned habitats, the current study was conducted to evaluate the effects of RIFA and habitat on survival of lone star and Gulf Coast ticks. Within treatments (burned habitat with RIFA, burned habitat without RIFA, and unburned habitat without RIFA), 10 tick enclosures were installed and seeded with engorged lone star or Gulf Coast tick nymphs. After molting, ticks within enclosures were collected. Survival of lone star ticks in burned habitats (regardless of RIFA presence) was significantly lower compared with unburned habitat. Gulf Coast ticks had significantly greater survival in burned habitats (regardless of RIFA presence) compared to lone star ticks. In this study, burning status was more important for survival of ticks than presence of RIFA, with Gulf Coast ticks surviving better in burned habitat which typically experiences higher temperatures and lower humidity.

Keywords: *Amblyomma,* tick, red imported fire ant, fire, burning

Introduction

An increase in both the incidence and emergence of tick-borne diseases in the United States has been documented over the past several decades (Maeda et al. 1987, Parola et al. 2005, Goodin et al. 2006, Apperson et al. 2008). For example, diagnosed cases of Lyme disease, ehrlichiosis, and Rocky Mountain spotted fever have increased greatly in the past 30 years (Fishbein et al. 1984, White et al. 1991, Dalton et al. 1995, Orloski et al. 2000, Openshaw et al. 2010). Furthermore, diseases such as Southern tick-associated rash illness (STARI) (causative agent currently unknown), American boutonneuse fever (*Rickettsia parkeri*), and babesiosis (not caused by *Babesia microti*) have emerged in the last ten to fifteen years (Masters et al. 1998, James et al. 2001, Paddock et al. 2004, Chauvin et al. 2009). Due to these trends, it has become increasingly important to identify ways in which humans can reduce risk of tick-borne diseases. Specifically, identifying ways to reduce entire tick populations that are financially viable and easily applied on a landscape-level would be highly desirable and effective in reducing risk of human tick-borne diseases. Perhaps one of the most promising methodologies fitting these criteria is prescribed burning.

There is general agreement that prescribed fire initially causes a decrease in tick populations, with the extent of the decrease correlating with intensity of burn (Hoch et al. 1972, Stafford et al. 1998). However, there is discrepancy regarding the long-term effects of burning on tick populations. While some studies have documented long-term decreases in tick populations (Jacobson & Hurst 1979, Drew et al. 1985, Wilson 1986, Cully 1999), others documented increased tick populations (Mather et al. 1993, Cilek and Olson 2000) while others found that tick populations simply re-bounded to pre-burn population levels

within approximately one year of a burn (Davidson et al. 1994, Stafford et al. 1998, Cully 1999). However, these studies often failed to account for potential confounding factors that are known to affect tick populations such as host abundance, microclimate and vegetation structure. Furthermore, these studies typically failed to simulate operational management conditions in which prescribed fire would be utilized over large areas (hundreds to thousands of hectares) and repeated on a regular cycle, typically every two to five years. This later point is of particular importance as one of the primary purposes of regular prescribed burning is to physically alter habitat. Specifically, long-term prescribed fire creates a unique habitat and vegetation structure characterized as having a dense herbaceous understory, reduced mid-story and a semi-open canopy.

Interestingly, red imported fire ants (RIFA)(*Solenopsis invicta)* are known to prefer sites that receive direct sunlight and are often associated with disturbed habitat (Anonymous 1958, Hung and Vinson 1978, Howard and Oliver 1979, Tschinkel 1986, Jemal and Hugh-Jones 1993). Indeed, RIFA are commonly found in burned and/or open habitats and are less common in fully forested, unburned habitat (Anonymous 1958, Green 1967, Fleetwood et al. 1984). Furthermore, there have been studies that suggest RIFA could reduce lone star tick, *Amblyomma americanum,* (L.) populations under experimental field settings (Harris and Burns 1972, Burns and Melancon 1977). For example, Harris and Burns (1972) found that nymphs and adults of lone star ticks released at sites with RIFA had significantly lower survival than when released at sites that had been treated with mirex bait to eliminate RIFA. Additionally, the predatory role of fire ant species on ticks has been shown in several studies (Fleetwood et al. 1984, Barré et al. 1991). For example, Fleetwood et al. (1984) found that RIFA were the most

common predators of lone star ticks in their study sites inexas. Interestingly, some metastriate ticks, such as *Amblyomma* spp., produce an ant-diversionary secretion from their large wax glands (Yoder et al. 1993). Such an adaptation would seem to indicate the possibility that predatory ants are acting as an important selective force on these metastriate ticks. Nevertheless, data are too scant to definitively show the impacts of RIFA on tick populations, especially tick species other than the lone star tick.

During a recent study in southwestern Georgia, tick populations were significantly reduced in areas subjected to long-term prescribed burning as compared to unburned habitat (E.R.G., unpublished data). Furthermore, tick species composition differed between burned and unburned sites, with lone star ticks preferring unburned sites and Gulf Coast ticks, *A. maculatum*, Koch preferring burned habitats. However, it was not determined if presence of RIFA, habitat differences and/or some other unknown factor was causing this shift in species composition and population declines observed in burned areas. Therefore, the current study was designed to identify the impacts of RIFA and habitat type created by the presence or absence of long-term prescribed fire on the survival of lone star ticks and Gulf Coast ticks. It was hypothesized that lone star ticks would have reduced survival in the burned habitats as compared to the Gulf Coast ticks. Furthermore, it was hypothesized that RIFA would result in reductions of both tick species.

Materials and Methods

Study Area

This study was performed in 2011 at the Joseph W. Jones Ecological Research Center at Ichauway (hereafter, Ichauway). Ichauway is in Baker County, southwestern Georgia, 20 km south of Newton, Georgia, USA. Ichauway is a 12,000-ha research site dominated by mature long-leaf pine forest, with approximately 24 km of the Ichawaynochaway Creek and 22 km of the Flint River running through or along the border of the site. Prescribed fire management has been utilized and meticulously recorded for 90 years at Ichauway. Biennial burns are typically used throughout the long-leaf pine forests.

For this particular study, we utilized sites with two different habitat types: 1) subjected to long-term prescribed burning and 2) no recorded prescribed burning. Burned habitat had been subjected to biennial prescribed burning since institution of fire management at Ichauway and was characterized as long-leaf pine forest. Specifically, burned habitat was comprised of two layers, a relatively open long-leaf pine overstory and an understory composed primarily of wiregrass (*Aristida beyrichiana*) as well as various forbs, grasses, and low woody species.

A site with no recorded prescribed burning was located within a riparian area along the Ichawaynochaway Creek. Riparian areas have historically not been burned at Ichauway. Unburned, riparian habitat is characterized as lacking an understory, having a fairly dense midstory and a closed, hardwood canopy.

Study Design

RIFA and habitat treatments. Because habitats that have been regularly subjected to prescribed fire differ greatly from unburned areas, we identified the effects of both fire treatments (burned and unburned habitat) and RIFA presence or absence on survival of lone star and Gulf Coast ticks. To do this, three treatment areas were established, each 100m x 100m: burned habitat with RIFA, burned habitat without RIFA and unburned habitat without RIFA. A full factorial design was not used because long-term absence of fire tends to make habitat unfavorable for RIFA and no unburned habitat with RIFA was present at Ichauway (Fleetwood et al. 1984, Tschinkel 1993). However, the unburned, RIFA-absent treatment occurs naturally; therefore, we chose an unburned area approximately 6.4 km from the burned treatments for this aspect of the study.

Burned habitat had not been burned in approximately two years. Because RIFA naturally occurs in burned habitat, the RIFA-present treatment was not further treated and ten RIFA mounds were identified on which tick enclosures would later be placed. To confirm RIFA mound identification, mounds were minimally disturbed and ants exiting the mound were identified through defensive behavior and morphologic characters. The length, width and height of each mound were then measured.

To eliminate RIFA from the burned, RIFA-absent treatment, transects were walked covering the entire treatment area. This was done when air temperatures were between 22.0 and 32.0°C which are conducive to ant activity (Cokendolpher and Francke 1985, Porter and Tschinkel 1987). All active RIFA mounds were visually identified as described above and then eliminated by inserting a metal rod and introducing boiling water into the colony as in Tschinkel and King (2007). To ensure that colonies were not

re-established, surveys for and elimination of mounds were performed once a week during the length of the study.

RIFA Collections. To confirm that RIFA were active and able to gain access to enclosures in the burned, RIFA-present treatment, ants were randomly collected from a minimum of seven tick enclosures after collection of Gulf Coast ticks and immediately prior to introduction of lone star ticks . Collections of ants were performed during dry weather and during temperatures in which RIFA are known to be active (22-32°C). Ants were collected by placing hotdog-baited test tubes directly in the tick enclosures for 20 minutes (Kafle et al. 2008). After 20 minutes, tubes were sealed with any ants inside being captured. Ants were frozen at -20°C until they could be enumerated and morphologically identified under a dissection microscope.

Tick Enclosures. To ensure that ticks were able to utilize normal anti-predatory and moisture retention behaviors, 30 tick enclosures were constructed that would allow both ticks and ants to be free within a small portion of their respective treatment areas. Tick enclosures were made from 55 gallon, white plastic barrels that were 61 centimeters in diameter. Barrels were cut in half (each half was an enclosure) and the ends of the barrels were cut off and used as lids for the enclosures. To allow for air flow and penetration of sunlight and precipitation, eight 5-inch holes were placed on the sides of the barrel and one hole which encompassed approximately 60% of the lid. All holes were covered with 24 wires per inch screen and sealed with plastic silicone.

Ten enclosures were installed in each treatment. Edges of enclosures were buried approximately 3 cm into the ground. In RIFA-absent sites, enclosures were placed every 10 m on a 90 m transect in the center of the plots. In the RIFA-present site, tick

enclosures were placed where RIFA mounds were present. For mounds smaller than 1,400 sq cm (n=2), enclosures were placed over the entire mound. For mounds greater than 1400 sq cm (n=8), enclosures were placed in the mounds, with approximately 30% of the mound being within the enclosure.

Tick Survival. Two randomly assigned ground litter control samples, 69 centimeters in diameter, were collected in each treatment area prior to each tick species being placed in the enclosures to check for presence of ticks in the area. In addition, the entire treatment area was flagged to confirm the absence of ticks in the area.

Gulf coast tick nymphs were placed in enclosures in early March and lone star tick nymphs were placed in tick enclosures in mid-June which corresponds with normal nymph activity in this region (E.R.G., unpublished data, Cilek and Olson 2000). Thus, the two tick species did not co-exist in the enclosures. Simulating tick activity periods ensured that tick mortality was not confounded by 1) ticks being subjected to weather conditions in which they are not typically active or 2) potential interspecies competition that does not normally occur in the wild.

Laboratory-reared engorged nymphs were acquired from Oklahoma State University (Stillwater, OK) and 19 Gulf coast nymphs and 17 lone star nymphs were introduced into each tick enclosure at their respective times. Slight differences in numbers between lone star and Gulf coast ticks were due to differences in availability during the study period. Engorged nymphs were placed in the center of the enclosures and the lid secured with bricks and sealed shut with tape.

As a control for molting time, 15 Gulf Coast nymphs and 20 lone star nymphs from the same batch were placed in a laboratory tick chamber and held at ~80% humidity

and 25° C. An additional 16 Gulf coast nymphs and 20 lone star nymphs were placed in an enclosed mesh bag within a metal cage that was located in the burned, RIFA-absent treatment. The mesh bag was partially covered with ground-litter found in the area to simulate field conditions.

Tick enclosures were left undisturbed while ticks molted, with the exception of seal inspections and general condition of the enclosures being checked twice weekly. Collection of molted Gulf Coast tick adults commenced once the final tick had molted in the outdoor control (ticks in the indoor tick chamber had completed molting approximately seven days prior to the outdoor control molt completion). Unfortunately, all ticks in the lone star tick outdoor control bag escaped. Based on data from Gulf Coast ticks, it was determined that lone star tick collections would begin eight days after the final tick in the indoor control had molted.

To collect ticks, lids were unsealed and removed. All vegetation, ground litter and topsoil was removed by hand and meticulously checked for ticks. All adult ticks, dead nymphs and molt-casings were saved in 70% ethanol for later identification and enumeration. General observations of behavior and detailed locations of ticks were noted. Collections of all enclosures for Gulf Coast and lone star ticks were completed in thirteen and ten days respectively. Collections were alternated between sites to ensure that differences in collection dates did not affect treatment outcome. Each tick enclosure was re-located immediately adjacent to its original location after collection of the Gulf Coast ticks to provide undisturbed habitat for the lone star tick experiment.

Statistics

An Analysis of Variance (ANOVA) (PROC GLM, SAS Institute 2010) was used to determine whether habitat affected survival of each tick species differently, with tick species and RIFA presence or absence being treated as main factors in a factorial analysis. An LSD mean separation procedure was used to determine differences between treatments. An ANOVA was also run to determine whether RIFA affected survival of either tick species, with tick species and RIFA presence/absence being treated as main factors in a factorial analysis. Because we did not have a fully factorial design due to the lack of the unburned, RIFA-present treatment, we analyzed the effect of three treatments (burned, RIFA-present; burned, RIFA-absent; & unburned, RIFA-absent) on survival of each tick species within a one-way ANOVA. An LSD mean separation procedure was used to determine differences between treatments when applicable.

Results

Tick Survival

No ticks were found in the two ground litter control samples taken in each treatment prior to the introduction of each tick species. A single, flat Gulf Coast tick nymph was collected while flagging in the burned, RIFA-present exclosure prior to introducing Gulf Coast ticks to their enclosures. No other ticks were collected when flagging sites prior to introducing either tick species.

When enclosures were searched at the end of the study, no live engorged nymphs were found. For Gulf Coast ticks on average 3.1 ± 1.1 ($\bar{x}$ ± SE), 2.5 ± 0.7, and 5.8 ± 1.6 molt casings per enclosure were found in burned, RIFA-present; burned, RIFA-absent,

and unburned, RIFA-absent respectively. For lone star ticks, only a single molt casing was found in one of the enclosures in the unburned, RIFA-absent treatment. One dead Gulf Coast nymph and 1 dead lone star nymph were found in the burned, RIFA-present and unburned, RIFA-absent treatments, respectively.

Average molt success and survival for Gulf Coast ticks was highest in the two burned treatments (Figure 1) regardless of RIFA presence with 35.3 ± 7.4%, 42.1 ± 7.3%, and 31.6 ± 4.6% surviving in burned, RIFA-present; burned, RIFA-absent; and unburned, RIFA-absent groups, respectively. Molt success and survival for lone star ticks was highest in the single unburned treatment group (25.5 ± 6.2%) and was very low in the two burned groups (0.6 ± 0.6% and 5.9 ± 3.9% (burned, RIFA-present and burned, RIFA-absent, respectively) (Figure 5.1). There was no interaction between tick species and RIFA in regards to tick survival ($F = 0.02$; $df = 1, 35$; $p = 0.8844$,). Presence and absence of RIFA had no effect on survival of either tick species ($F = 1.32$; $df = 1, 35$; $p = 0.2586$).

There was a significant interaction between tick species and habitat created by fire ($F = 7.86$; $df = 1, 34$; $p = 0.0083$), indicating that habitat associated with long-term prescribed fire impacted the two tick species differently. While there was no significant difference in survival of the Gulf Coast tick across all 3 treatments ($F = 0.69$; $df = 2, 26$; $p = 0.5083$), there was a significant difference in survival of lone star ticks ($F = 9.99$; $df = 2, 26$; $p = 0.0006$). Furthermore, there was a significant difference between the survival of the two tick species ($F = 45.01$; $df = 1, 35$; $p < 0.0001$). Specifically, survival of Gulf Coast ticks was greater than survival of lone star ticks within burned habitats ($p < 0.0001$). Not surprisingly, survival of lone star ticks was significantly greater in the unburned habitat versus the burned habitat ($p = 0.0144$). Interestingly, however, no

significant differences in survival between the Gulf Coast ticks and lone star ticks in the unburned habitat were observed ($p = 0.4287$). While lone star ticks survived best in unburned habitats, their survival in this habitat was still significantly lower than the survival of Gulf Coast ticks in the burned habitat ($p = 0.0404$).

Ant Collections in the RIFA Present Treatment

During the sample performed in early May immediately after *A. maculatum* adults were collected, an average of 100.6 ± 51.3 RIFA were collected. No other ant species were collected. For the RIFA collection performed in mid-June prior to introduction of *A. americanum* nymphs into enclosures, an average of 178.4 ± 53.2 RIFA were collected. Four *Paratrechina* sp. were also collected from a single enclosure.

Discussion

The goal of our study was to determine if RIFA and/or habitat associated with long-term prescribed fire affected survival of two common tick species in the southeastern US. Neither RIFA presence/ absence nor habitat associated with prescribed fire reduced survival of Gulf coast ticks, but we did detect a significant negative effect of habitat resulting from prescribed fire on the survival of lone star ticks. Although RIFA are known to feed on and kill ticks(Harris and Burns 1972, Burns and Melancon 1977), seasonal activity differences among and between tick species and life stages may allow some species and/or life stages to avoid peak RIFA activity as RIFA activity is also seasonal (Cokendolpher and Francke 1985, Porter and Tschinkel 1987, Cilek and Olson 2000, E.R.G., unpublished). Thus, non-synchronous activity between ticks and RIFA could impact the survival of tick species and life stages differently.

While we did not detect a significant impact of RIFA on lone star tick survival, we did not have an unburned, RIFA-present treatment and survival of lone star ticks was extremely low in the burned habitats. Thus, it is suggested that further studies need to be performed to better evaluate the impacts of RIFA on lone star ticks. Indeed, while our results on the impact of RIFA on lone star ticks within burned areas were not significant, we observed lower survival in the RIFA present treatment and it is known that RIFA feed on lone star ticks and have, in cases, been correlated with significant declines in lone star tick populations (Harris and Burns 1972, Burns and Melancon 1977, Fleetwood et al. 1984).

The finding that lone star tick survival was reduced in burned habitat, regardless of RIFA presence, has important implications for disease management and control. Lone star ticks are known vectors of several zoonotic pathogens, including *E. chaffeensis*, *E. ewingii,* and the causative agent of STARI. This tick species is widespread, utilizes a wide host range, and occurs at high densities in the southeastern United States (Childs and Paddock 2003). Our findings suggest that prescribed burning on a regular basis (approximately every 2 years) results in a habitat unsuitable for the survival of lone star ticks and thus could potentially be used for reduction of this tick species. Future studies should investigate the impacts of prescribed fire on lone star ticks on a population level.

Although Gulf coast tick survival was similar across all groups, higher numbers of ticks survived in the two burned treatments and significantly more Gulf coast ticks survived in burned habitats relative to lone star ticks. These data suggest that Gulf Coast ticks are better adapted to surviving in burned habitats than lone star ticks which is supported by work by Needham (1991) who showed that Gulf Coast ticks have lower

whole-body permeability and therefore can retain moisture better than lone star ticks. Because burned habitats receive more direct sunlight due to their semi-open canopies and lack of mid-story, it appears that the ability of Gulf Coast ticks to retain moisture more so than the lone star ticks allows them to better avoid death by desiccation. Additionally, a large number of Gulf Coast tick adults recovered from enclosures in burned habitats were found in locations where moisture would be highly conserved (e.g., under the bark or inside rotting sticks), perhaps indicating that moisture retention may also be aided by behavior. Conversely, very few lone star ticks were found alive in the burned habitat and those that were found were typically on the surface of the soil or on vegetation where relative humidity would be low.

Within the two burned groups, fewer Gulf Coast and lone star ticks survived in the RIFA-present group. Although these results were not significant, effects of RIFA on either tick species may be biologically significant. This study was done on a small scale and it is possible that ant predation could impact ticks if examined at a larger scale. Importantly, it is also possible that the time period of tick activity is a major factor regarding possible RIFA predation. Gulf coast tick nymphs in southwestern Georgia and northwestern Florida are primarily active from February-April (Cilek and Olson 2000, E.R.G, unpublished data), whereas lone star tick nymphs are most active in May-June (E.R.G. unpublished data). RIFA activity is affected by temperature, with activity dropping during cold months and increasing during warmer months (Cokendolpher and Francke 1985, Porter and Tschinkel 1987). In Tallahassee, Florida (120 km south of our study site), Porter and Tschinkel (1987) found that RIFA activity increases significantly between March and July, with activity peaking in July. Indeed, our data seem to

corroborate this as fewer ants were captured in the RIFA present treatment in early May (immediately after Gulf Coast tick collections) as compared to in mid-July (immediately prior to lone star tick releases). Thus, it is possible that lone star nymphs experience greater RIFA predation because their peak activity period overlaps with RIFA activity, whereas Gulf Coast tick nymphs are active when RIFA are less active. Furthermore, it may also be possible that anti-ant secretions produced by some *Amblyomma* sp. (Yoder et al. 1993) may differ between the Gulf Coast tick and lone star ticks, thus accounting for part of the differential mortality between the tick species in response to RIFA predation. While the nymphs only comprise one portion of the tick's life cycle, the impact or lack of impact of RIFA on a particular life stage of a species of tick would directly affect abundance of future life stages.

In conclusion, long-term use of prescribed fire may be a useful tool for the reduction of tick populations and tick-borne disease risk; however, additional studies at more sites and for multiple seasons are needed to determine the long-term impact of prescribed fire on tick populations. Long-term prescribed burning directly alters habitat that likely results in a microclimate that is not conducive to lone star tick survival, the most abundant tick in much of the southeastern US (Merten and Durden 2000, Paddock and Yabsley 2007). In contrast, changes in habitat associated with long-term prescribed burning increased the survival of Gulf Coast ticks which have been previously shown to be capable of better moisture retention than lone star ticks. Finally, while impacts of RIFA on lone star ticks are not clear due to their extremely low survival in both burned habitats, RIFA did not have a significant effect on the survival of Gulf coast ticks. However, the decreased survival of ticks when RIFA were present may be important

biologically and follow-up studies that investigate larger-scale impacts or are able to address the effects of RIFA on lone star tick survival may still be useful to better determine the biological significance of RIFA on tick populations.

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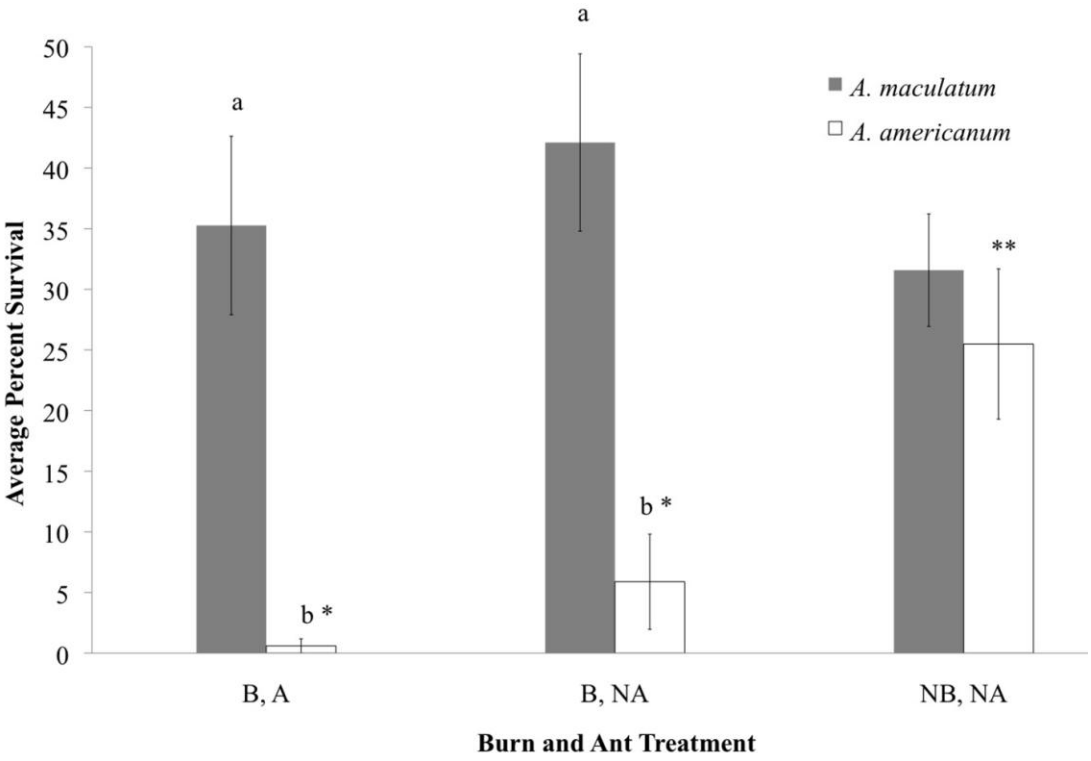

Fig 5.1. Effects of habitat and RIFA presence on survival of *A. americanum* and *A. maculatum* nymphs to adulthood. B = burned habitat, A = RIFA present, NA = No RIFA, NB = unburned habitat. Bars with different letters indicate statistical significance ($p < 0.05$). Bars with different numbers of asterisks indicate that they are significantly different from one another ($p < 0.05$).

CHAPTER 6

DETECTION OF A NOVEL SPOTTED FEVER GROUP *RICKETTSIA* IN THE GOPHER TORTOISE TICK, *AMBLYOMMA TUBERCULATUM* (USA)*

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Abstract

The gopher tortoise tick, *Amblyomma tuberculatum*, is distributed throughout the southeastern United States (US), and its immature life stages have been reported to occasionally bite humans. Here we report detection of a novel spotted fever group (SFG) *Rickettsia* in *A. tuberculatum* ticks collected in the southern US. Among questing ticks collected in Georgia, 10 pools of larvae were identified as gopher tortoise ticks, *A. tuberculatum.* Each of these samples was positive for SFG Rickettsiae. The RFLP profiles were identical to each other, but distinct from those of other rickettsiae previously found in *Amblyomma* spp ticks. Partial genetic characterization of the novel agent was achieved by sequencing the 17kDa, *glt*A, *omp*B, *omp*A, *rpo*B, and *sca*4 genes. Analysis of a concatenated tree of four genes (*glt*A, *omp*B, *omp*A, and *sca*4) demonstrates close relatedness of the detected *Rickettsia* to several SFG *Rickettsia* spp. The identical rickettsial DNA was detected in 50% and 70% of adult *A. tuberculatum* ticks from Mississippi and Florida respectively. The results indicate wide distribution of a novel *Rickettsia*, capability for transovarial transmission, and high prevalence in tested tick populations.

Key words: *Amblyomma tuberculatum*, SFG *Rickettsia*, phylogenetic analysis.

Introduction

In the United States (US), *Amblyomma americanum* and *A. maculatum* attract major attention as the most prevalent human-biting tick species (Stromdahl et al. 2011) and are known to harbor *Rickettsia amblyommii* and *R. parkeri*, respectively (Castellaw et al. 2010, Paddock et al. 2010). However, other species of the genus *Amblyomma* also can

attack humans and could potentially contain tick-borne pathogens. The gopher tortoise tick, *A. tuberculatum*, is found in Florida, Georgia, South Carolina, and Mississippi (Goddard 2002, Williams et al.1999). As *A. tuberculatum* larvae are occasionally found on people (Goddard 2002), this species may be considered as a human-biting tick. Here we report detection of a novel spotted fever group *Rickettsia* in *A. tuberculatum* ticks collected in Georgia, Mississippi, and Florida.

Materials and Methods

In the course of a CDC-UGA collaborative field study in southern Georgia (Baker Co.), ticks were collected from all sources including people and trapped animals. Over 200 larvae removed from a person`s clothes in 2009 were identified as *A. tuberculatum* and tested for the presence of rickettsiae. Additionally, 20 samples, each containing 2 legs from individual alcohol-preserved voucher specimens of *A. tuberculatum*, were obtained from the Mississippi Museum of Natural Science, and 68 alcohol-preserved adult *A. tuberculatum* ticks were obtained from the University of Florida, College of Veterinary Medicine (Table 6.1). Prior to testing for the presence of spotted fever group (SFG) rickettsiae, adult ticks were identified using morphological keys (Cooney and Hays, 1972). Species identity of larval and adult ticks was additionally confirmed by sequencing of the 12S mitochondrial rDNA (Beati and Keirans 2001).

DNA was extracted from 10 pools of larvae and individual adult tick samples using the Qiagen DNEasy Blood and Tissue kit (Qiagen Inc., Valencia, CA) according to the manufacturer`s protocol of extracting DNA from tissues. Sample preparation included grinding ticks previously frozen in liquid nitrogen. Samples were tested for the presence

of spotted fever group rickettsiae based on the amplification of a 114 bp fragment of the rickettsial gene encoding the 17 kDa protein using genus-specific primers as follows. Two microliters of extracted DNA was combined with core reagents of a Stratagene Brilliant kit (Agilent Technologies, La Jolla, CA) in a 20 ul reaction mixture containing primers R17K135F (ATG AAT AAA CAA GGK CAN GGH ACA C), R17K249R (AAG TAA TGC RCC TAC ACC TAC TC) each at final concentration of 10 uM and a probe R17KBC (5TTG GTT CTC AAT TCG GTA AGG GTA AAG G3 - 5' Cal Red 610 3' BHQ2) at final concentration of 80 uM. The PCR was performed on an iCycler iQ (Bio-Rad) using the following thermal cycler parameters: activation at 95°C for 8 min, followed by 40 cycles of 95°C for 15 s, 57°C for 60 s, followed by a 4°C hold. Genomic DNA from *Rickettsia amblyommii* was used as a positive control. Two no template controls (distilled water) were included on each plate. Tick extracts positive for SFG rickettsia were subjected to Restriction Fragment Length Polymorphism (RFLP) analysis as described previously (Eremeeva et al. 1994). Multiple locus sequence typing analysis of *Rickettsia* gene fragments included partial sequences of 17kDa (Anderson and Tzianabos 1989), *glt*A (Labruna et al. 2004, Roux et al. 1997), *omp*B (Roux and Raoult 2000), *omp*A (Roux et al. 1996), *rpo*B (Paddock et al 2010), and *sca*4 (Sekeyova et al. 2001) genes. An ABI PRISM 3.0 BigDye Terminator Cycle Sequencing kit (Applied BioSystems, Foster City, CA) was used for performing sequence reactions as recommended by the manufacturer. The amplicons were purified using the Wizard SV gel and PCR clean-up system (Promega, Madison, WI) and sequenced on an Applied BioSystems 3130*xl* genetic analyzer.

Sequences were assembled using the DNASTAR Lasergene 8 software package. Homologous sequences were detected using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Sequence Tool Search Engine (BLAST). DNA sequences of closely related SFG rickettsiae representing validated and "*Candidatus"* species from NCBI GenBank were included in the analysis. Sequence alignments were performed using ClustalW, and individual phylogenetic trees were drawn using MEGA4.0 software (Tamura et al. 2007). Four genes were concatenated (*glt*A-*omp*A-*sca*4-*omp*B) and a total of 4,663 bp were analyzed. Species for which analyzed sequences were unavailable could not be included into the concatenated tree. The nucleotide sequences generated during this study were deposited in the NCBI GenBank under the following accession numbers: JF934882 for the 17-kDa protein gene fragment, JF934883 for the *glt*A fragment, JF934880 for the *rpoB*, JF934878 for the *omp*A fragment, JF934879 for *omp*B, and JF934881 for *sca*4.

Results

All 10 larval pools of *A. tuberculatum* from the southern Georgia contained DNA of a SFG *Rickettsia,* and the sequence of 17 kDa protein gene was identical in all of them. Adult *A. tuberculatum* ticks from Mississippi and Florida contained rickettsial DNA with the prevalence of infection 50% and 70% respectively. *Omp*A gene sequences of *Rickettsia* detected in ticks from Mississippi and Florida were identical to those from South Georgia. Prevalence of infection in ticks from the two states did not differ significantly (P=0.0716).

Analysis of the DNA sequences revealed that the most conserved 17 kDa, *glt*A and *rpo*B genes of the *Rickettsia* present in *A. tuberculatum* ticks showed the highest BLAST identity to the core SFG rickettsiae. The 1,181 bp *glt*A fragment of the novel *Rickettsia* demonstrated 100% identity to that of *R. sibirica sibirica* and 99% to other core SFG rickettsiae (>25 sequences), with 1 to 6 single-nucleotide polymorphisms (SNPs). The *gltA* sequence of the new *Rickettsia* also showed 100% identity to that of *Rickettsia* sp. Atlantic rainforest and *R. parkeri* strain ApPR which were identified as closely related to *R. parkeri* Maculatum 20. Sequence of the *rpoB* gene (477 bp) fragment was 100% identical to the homologous gene of *R. sibirica mongolotimonae* and 99% identical to *R. rickettsii*, *R. africae, R. conorii, R.parkeri, R. sibirica sibirica, R. peacockii, R.honei, R.slovaca,* and *R. massiliae*. Partial sequences of *omp*B, *sca*4 and *omp*A genes of the detected *Rickettsia* demonstrated similar relatedness to the homologous genes of the core SFGR but with lesser degrees of identity: 97-99%, 97-98% and 96-99% respectively. Comparison of *omp*A gene sequence of the new *Rickettsia* to a recently described rickettsial endosymbiont of *A. maculatum* showed only 92% of identity (Paddock et al. 2010). All individual phylogenetic trees constructed for each gene had, in general, a similar branch structure to the concatenated tree (Figure 6.1).

The RFLP analysis of the fragment of the *omp*A gene was performed for the novel *Rickettsia* as well as for *R. amblyommii* and *R. parkeri* as the most common rickettsial agents in *Amblyomma* spp. ticks in southern US. The RFLP pattern of the novel *Rickettsia* in combination of RR190.70-RR190.701 primers and *Pst*1 enzyme was similar to that of *R. parkeri* (Figure 6.2). Differentiation of the detected *Rickettsia* from either *R.*

amblyommii or *R. parkeri* was obvious in RFLP combinations RR190.70-RR190.701 with *Rsa*l (Figure 6.2) as well as in combination RR190.70-RR190.602 with *Pst*1 & *Rsa*l.

Discussion

Overall, a unique DNA sequence of a novel *Rickettsia* sp. has been detected in unfed larvae of *A. tuberculatum* removed from a person`s clothes from the southern Georgia and partially characterized. The same agent was identified in adult *A. tuberculatum* ticks collected in Mississippi and Florida. To our knowledge, this is the first report of a *Rickettsia* sp. in the gopher tortoise tick, *A. tuberculatum*. Based on the recommended criteria for naming of new rickettsiae (Raoult et al. 2005), at present, there is not enough data to designate the detected *Rickettsia* as a new species as the organism has not been isolated in cell culture. Therefore, only a *Candidatus* name "*Rickettsia* sp. from *A. tuberculatum"* can be designated at present time. The detected *Rickettsia* is closely related to several SFG rickettsiae pathogenic to humans, including *R. parkeri*, *R. conorii,* and *R. africae*. Our results demonstrate wide geographical distribution of the "*Rickettsia* sp. from *A. tuberculatum"* and a high prevalence within tested populations of the gopher tortoise tick. Detection of the "*Rickettsia* sp. from *A. tuberculatum"* in unfed larvae indicates its capability for transovarial transmission. However, neither the ability of this *Rickettsia* for horizontal transmission, nor its pathogenicity and immunogenicity in vertebrate hosts are known at this time. Considering that *A. tuberculatum* may occasionally bite humans (Goddard 2002), further studies on virulence and pathogenicity of the new *Rickettsia*, as well as the potential for human exposure, are warranted.

Acknowledgements

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Table 6.1. Tick collections and the prevalence of infection with newly detected *Rickettsia* sp. among *A. tuberculatum*.

Locality	Host	Collection date	Number of ticks tested	Prevalence of infection
Baker County, GA	Questing ticks	Fall 2008-Spring 2009	10 pools*20 LL	100%
Wayne County, MS	*Gopherus polyphemus*	05/2008 05-08/2009	20 voucher specimens (2 legs in each)	50%
Brevard County, FL	*Gopherus polyphemus*	05-07/2006	68 alcohol-preserved individual ticks	70%

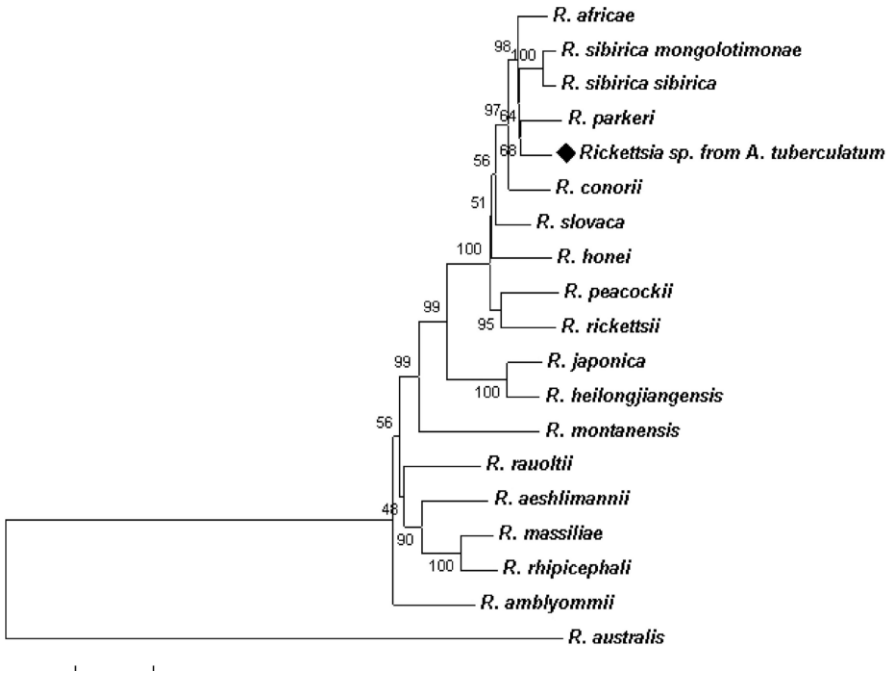

Figure 6.1. The phylogenetic position of novel SFG *Rickettsia* from *A. tuberculatum* tick inferred using the Neighbor-Joining method. Four gene sequences (*glt*A-*omp*A-*sca*A-*omp*B) were concatenated and a total of 4,663 positions were analyzed. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The percentahe of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion options). Bar = 1% nucleotide sequence divergence. Phylogenetic analyses were conducted in MEGA4.

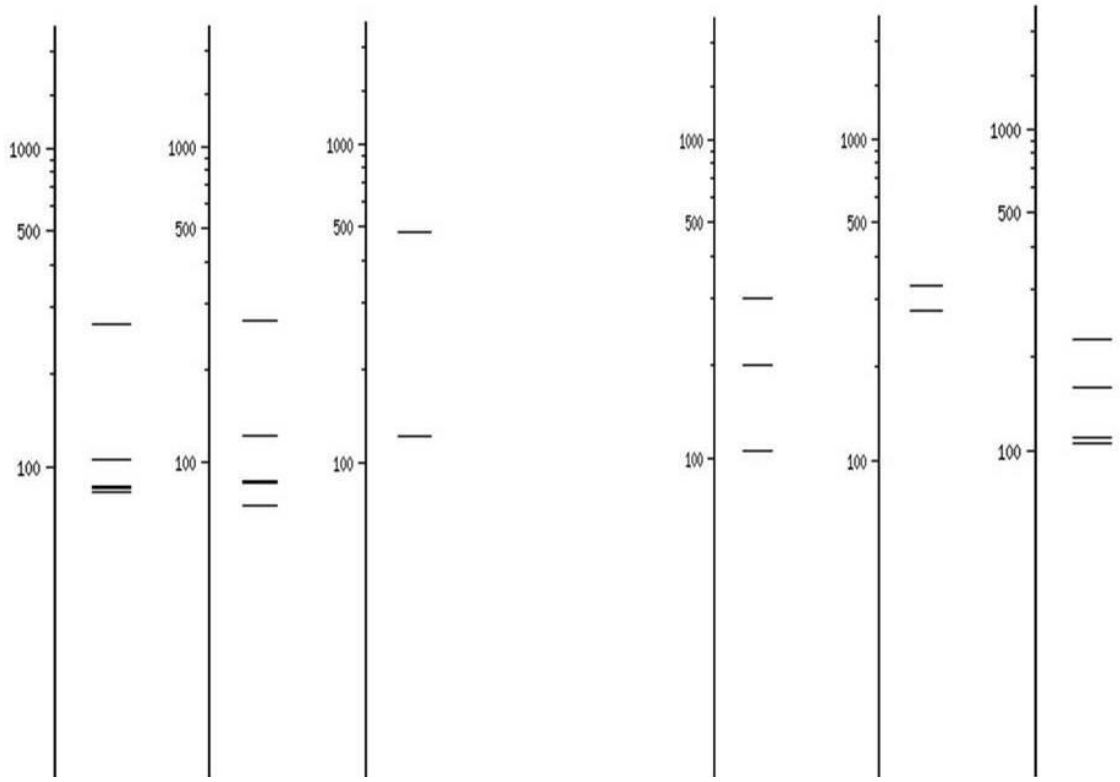

Figure 6.2. RFLP patterns of *R. parkeri/"R. amblyommii"/ "R* sp. from *A. tuberculatum*" *omp*A gene, RR 190.70-701, digested with Rsa1 enzyme. RFLP patterns of *R. parkeri/"R. amblyommii"/ "R* sp. from *A. tuberculatum*"*omp*A gene, RR 190.70-701, digested with Pst1 enzyme.

CHAPTER 7

EPIDEMIOLOGIC FACTORS ASSOCIATED WITH INFESTATION AND PATHOGEN PREVALENCE IN TICKS PARASITIZING HUMANS IN GEORGIA, USA

Gleim, E.R., L.E. Garrison, D. Cole, M. Vello, M.Y. Savage, G. Lopez, R.D. Bergaus, and M.J. Yabsley. To be submitted to *Parasites and Vectors*.

Abstract

Background

The incidence and emergence of tick-borne diseases has increased dramatically in the United States during the past 30 years, yet few large-scale epidemiological studies have been performed on infested individuals. Epidemiological information, including disease development, may provide valuable information regarding effectiveness of tick bite prevention education, pathogen transmission, human disease dynamics, and potential implications for under reporting of tick-borne diseases. To gain insight into epidemiologic factors associated with tick infestation in Georgia (USA), the current study was undertaken in which ticks found attached to residents were submitted for identification and polymerase chain reaction (PCR) testing for *Ehrlichia*, *Anaplasma*, *Borrelia*, and *Rickettsia* spp. Attempts were made to interview tick bite victims immediately after submission and three weeks after the tick bite to identify various epidemiologic factors associated with infestation and if signs suggestive of a tick-borne disease developed.

Results

From April 2005-December 2006, 444 participants submitted 597 ticks (426 *Amblyomma americanum*, 142 *Dermacentor variabilis*, 19 *A. maculatum*, 7 *Ixodes scapularis*, 3 *Amblyomma* sp.) originating from 95 (60%) counties. *A. americanum* was most common in the Piedmont region and *D. variabilis* was most common in the Mountain and Piedmont regions. *A. americanum* was primarily attached to the trunk (52%) and legs (32%) whereas *D. variabilis* was primarily attached to the head (64%). Only 25 (34%) of 74 interviewed individuals purposely took tick bite prevention

measures. PCR positive ticks were attached to 136 participants. Of the 77 participants who developed illness, 27 were associated with positive tick(s). Of those 27 individuals, 12 fit the criteria for a possible tick-borne illness (i.e., tick attached >6 hours [if known], ≥4 day incubation period, and the individual exhibited clinical symptoms typical of a tick-borne illness without exhibiting cough, sore throat, or sinus congestion). Ticks from these individuals were positive for *R. amblyommii* (n=8), *E. ewingii* and *R. montana* (n=1), *R. montana* (n=1), *R. rhiphicephali* (n=1), and *Rickettsia* sp. TR-39 (n=1).

Conclusions

Although illnesses reported in this study cannot definitively be connected with tick bites, this prospective study provided data on tick infestation, pathogen prevalence, and epidemiologic factors associated with tick-infestation.

Key words: Epidemiology, tick-borne pathogens, tick-borne disease, *Rickettsia*, ticks, Georgia

Background

In the United States at least 11 species of ticks are vectors of pathogens of public health importance of which, two species (*Amblyomma americanum* and *Ixodes scapularis*) are responsible for the transmission of most known pathogens. For example, A. *americanum* transmits the causative agents of human monocytic ehrlichiosis (HME), *Ehrlichia ewingii* ehrlichiosis, and Southern Tick-Associated Rash Illness (STARI); whereas *I. scapularis* transmits the causative agents of Lyme disease, human granulocytic anaplasmosis (HGA), and babesiosis. Other tick-borne diseases of importance, particularly in the Southeast, include Rocky Mountain spotted fever (RMSF) and more

recently, American boutonneuse fever. While most of these tick-borne diseases are nationally notifiable to the Centers for Disease Control and Prevention, it is generally thought that tick-borne diseases are under reported due to misdiagnosis or failure to seek medical treatment, report disease, or identify specific disease agents (McQuiston et al. 1999, Parola et al., 2005, Diaz 2010). Furthermore, demographic and epidemiological information is infrequently collected on these patients who could prove to be useful in targeting high risk groups or behaviors (Loving et al. 1978, Felz et al. 1996, Harrison et al. 1997, Merten and Durden 2000, Stromdahl et al. 2001, Goddard 2002). A single study in which epidemiologic and demographic information was collected from tick-bite victims was performed in Kentucky (Murphree et al. 2009). However, there was a small sample size (n = 33) and study participants were geographically and demographically restricted to individuals eligible for health services at a single military base.

Additionally, because being bitten by an infected tick does not necessarily result in transmission of the pathogen and/or disease and those who do develop disease are not always reported, information on human encounter rates with infected ticks is nearly impossible to obtain. However, such data could assist in identifying geographic regions with high disease risk, as well as, provide insights into pathogen transmission dynamics. While several studies have been published regarding ticks parasitizing humans in the eastern US, submissions were sometimes received from a broad and varied geographic range and few tested for pathogens or obtained epidemiological information from the patients including whether disease ensued (Loving et al. 1978, Felz et al. 1996, Estrada-Peña & Jongejan 1999, Stromdahl et al. 2001).

To better describe the potential public health implications of human tick attachment, the current study was conducted. The specific objectives were to 1) Determine the geographic distribution and identity of ticks found attached to humans in Georgia, 2) PCR test ticks for suspected or known zoonotic pathogens, 3) collect demographic and tick exposure and bite risk data from tick submitters, and 4) Determine whether any submitters became ill during the 3 weeks following the tick bite.

Methods

Collection of Ticks and Epidemiological Data

From April 2005 – December 2006, a state-wide media campaign was performed requesting that residents with a tick(s) attached to them contact the Georgia Poison Center (GPC) to enroll in the study. Upon calling the GPC, it was verified that the tick had been attached to the participant and instructions for shipping the ticks to the Georgia Division of Public Health (GDPH) were provided. Once a submission was received, an epidemiologist from GDPH attempted to administer a telephone questionnaire to the submitter. Questions asked during the interview included the gender and race of the participant, county of residence, county in which the bite occurred, date of tick removal, estimated time of tick attachment, location of tick attachment on the body, activities performed and time spent outside, and interactions with domestic animals. Approximately 3 weeks later, a follow-up survey was administered during which the participant was asked to self-report any illness during the 3 weeks following the tick bite and if so, the date of onset, symptoms and medical treatment (if any) that were utilized. In 2006 only, participants were asked about protective measures taken against ticks (both

on purpose and not on purpose to avoid tick bites). Tick avoidance measures were divided into two categories, primary protection (e.g., things done to prevent ticks from attaching including, but not limited, use of DEET or other repellant, keeping extremities covered by clothing, and not sitting on the ground) and secondary protective measures (actions performed to find and remove ticks once attached including checking oneself frequently for ticks while outside, full body and/or buddy tick checks, and taking a shower).

Tick Identification and Pathogen Testing

All ticks were classified to species, life stage and gender morphologically using published keys (Kierans and Litwak, 1989; Kierans and Durden, 1998). Ticks were then homogenized and stored in phosphate buffered saline (PBS) and stored at -80°C until testing. DNA was extracted from 100µL of the homogenized tick solution using a Qiagen Viral RNA Minikit (Qiagen Science, Valencia, CA) as per the manufacturer's instructions. Testing ticks for bacterial agents was performed using several nested polymerase chain reaction (PCR) protocols which targeted the 16S rRNA genes of *E. chaffeensis* and *E. ewingii,* the 17kDa gene of *Rickettsia* spp., the flagellin gene (*fla*) of *Borrelia* spp., and the fopA gene of *F. tularensis*. Gene targets, primers, and cycling temperatures for organisms are indicated in Table 7.1.

For each primary reaction (except the Borrelia *fla* gene), the PCR reactions were assembled in 25 µL volumes containing 11 µL of molecular grade biological water (MGBW), 2.5 µL of $MgCl_2$, 5 µL of GoTaq Flexi Clear Buffer (Promega, Madison, WI), 0.25 µL of dNTPs (20 mM initial concentration), 0.5 µL of each primer (40 µM initial concentration), 0.25 µL of GoTaq Flexi (Promega, Madison, WI), and 5 µL of extracted

DNA. For the primary reaction targeting the *Borrelia fla* gene, the same volumes were utilized except, 10 µL of extracted DNA and 6 µL of MGBW were added to the reaction. For each secondary reaction, volumes of 25 µL were assembled containing 15 µL of MGBW, 2.5 µL of $MgCl_2$, 5 µL of GoTaq Flexi Green Buffer (Promega, Madison, WI), 0.25 µL of dNTP, 0.5 µL of each primer, 0.25 µL of GoTaq Flexi, and 1 µL of the primary PCR product.

Amplified products were separated using gel electrophoresis on a 2.5% agarose gel that was stained with ethidium bromide and visualized using a UV light. All amplicons for *Rickettsia* and *Borrelia* spp. were purified using a Qiagen gel extraction kit (Qiagen, Valencia, CA, USA) and bi-directionally sequenced at either the Integrated Biotechnology Lab at the University of Georgia (Athens, GA) or the Clemson University Genomics Institute (Clemson, SC). Resulting sequences were compared to published sequences in the GenBank database.

Precautions were taken to prevent and detect contamination including performance of primary and secondary reactions, and product analysis in distinct, designated areas. Negative controls were included in each DNA extraction and PCR reaction. Furthermore, positive controls for all agents were utilized during PCR reactions.

Statistical Analysis

For purposes of statistical analysis, when applicable, *E. chaffeensis*, *E. ewingii*, and *R. parkeri* were considered pathogens and all other *Rickettsia* species detected, other than *R. amblyommii*, were considered nonpathogenic organisms. Note that *R. rickettsii*, a known pathogen, was not detected in this study. *Borrelia lonestari* and *R. amblyommii* were analyzed separately as agents of unknown pathogenicity status. Counties were

designated as being in the Coastal, Coastal Plain, Mountain, or Piedmont georegion of the state. An exact test of independence was used to evaluate the significance of associations between tick species and georegion or site of attachment; use of tick bite prevention and gender or location at time of bite; and type of tick species or tick-borne pathogen prevalence and owning or working with animals. An exact test of independence was also used to evaluate the univariate associations between predictor variables and the probability of developing an illness. Predictors having a P-value less than or equal to 0.2 were included in a multivariable logistic regression model for the prediction of illness. Multivariable model selection proceeded by manual stepwise elimination until only variables with a P-value less than 0.05 remained. Variables considered in the multivariable analysis included whether the participant had a tick attached to them that was positive for a known pathogen, whether the person utilized secondary protective measures, and the amount of time the tick was attached to the participant.

Results

A total of 597 ticks (426 *A.* americanum, 142 *D. variabilis*, 19 *A. maculatum*, 7 *I. scapularis*, 3 *Amblyomma* sp.) (Table 7.2) were submitted by 444 participants (range of 1-17 ticks/person). These participants resided in 85 of the 159 (53%) counties in Georgia and were bitten in 95 (60%) counties (Figure 7.1). Submissions by county of residence (although not necessarily where the tick was obtained) ranged between 0.08 and 4.60 ticks per 10,000 county residents with Greene, Clay, Morgan, Harris and Chattooga counties having the highest submission rates (4.60, 3.07, 3.00, 2.86, and 2.72 ticks per 10,000 residents respectively) (Figure 7.1). In 2005, 483 ticks were submitted for which

the date on which they were removed was recorded, with *D. variabilis* adults and *A. americanum* adults and nymphs peaking in May which corresponded with peak enrollment in the study (Figure 7.2). Submission rates in 2006 were too low to evaluate seasonality of submissions with only 85 ticks being submitted in which date of removal was recorded.

For those participants that provided demographic data, 209 were male and 203 were female and 399 were Caucasian, eight were African American, four were multi-racial, and one was Asian. Georegion in which the tick was known to have attached to a participant was found to be significantly correlated with tick species ($p<0.001$), with *A. americanum* being most commonly detected in the Piedmont and *D. variabilis* being commonly detected in the Mountain and Piedmont regions (Figure 7.3). Other tick species were submitted too infrequently to evaluate regional associations.

Of the ticks submitted, the location of attachment on the body for 534 was reported. The location of attachment and tick species was significantly associated ($p<0.001$), with *A. americanum* primarily attaching to the trunk (52%) and legs (32%) followed by the head (11%) and arms (5%) and *D. variabilis* primarily attaching to the head (64%), followed by the legs (17%), trunk (15%), and arms (4%). Other tick species were not collected in large enough number to evaluate associations with attachment site.

Seventy-four participants were asked about protective measures taken against tick bites. Of these, 65 (88%) used some type of protection against ticks, although only 25 (34%) took some type of protection measure(s) to purposely prevent tick bites and/or remove ticks once attached. Primary protective measures were taken by 55 (74%) participants (12 specifically to avoid ticks) and secondary protective measures were taken

by 59 (80%) participants (25 to avoid ticks) while 49 participants (66%) utilized both primary and secondary protective measures. Primary protective measures (done on purpose or not) were not found to significantly reduce the chance of some type of illness after tick exposure ($p = 0.29$), while secondary protective measures did reduce the chance of illness ($p = 0.04$) (Table 7.2). Neither the participant's gender nor location at time of bite (i.e. at or away from one's home residence) significantly affected whether or not they purposely took preventative measures ($p = 0.24$ and $p = 0.20$ respectively).

One hundred and forty participants had at least one tick attached to them that was PCR positive for one or more species of bacteria. Georegion was found to be significantly correlated with the prevalence of non-pathogenic *Rickettsia* spp., with the Coastal region having the greatest prevalence (15%) ($p = 0.008$) followed by the Coastal Plain (11%), Mountain (10%), and Piedmont (4%) regions. No other correlations between georegion and bacteria prevalence were found, however, the prevalences of *R. amblyommii* by region (Coastal, Coastal Plain, Mountain, and Piedmont respectively) were 33%, 20%, 16%, and 25% and 4%, 0, 1%, and 1% for pathogenic bacteria. A single *B. lonestari* was detected in the Piedmont region. Two ticks were co-infected, one with *E. chaffeensis* and *R. amblyommii* and another with *E. ewingii* and *R. montana*. Furthermore, six participants had multiple ticks attached to them infected with *R. amblyommii*, one participant had multiple ticks infected with *R. montana,* one participant had one tick infected with *R. amblyommii* and another tick infected with *R. montana*, and one participant had one tick infected with *R. amblyommii* and another tick infected with *R.* sp. TR-39. A summary of the tick species and associated bacteria can be found in Table 7.3.

Fifty (18%) of the 282 individuals with no PCR positive ticks became sick (illness status of 32 additional participants with no PCR positive ticks could not be determined) within 3 weeks after their tick bite(s) as compared to 27 (21%) of 129 participants with PCR positive ticks. Specifically, 3 (60%) of 5 individuals with ticks positive for a pathogenic bacteria became sick as compared to 4 (18%) of 22 individuals with non-pathogenic *Rickettsia* spp, 19 (19%) of 100 individuals with ticks positive for *R. amblyommii*, and 1 (50%) of 2 individuals with ticks positive for *B. lonestari*. Participants with an attached tick positive for a known pathogen (n=5) were significantly more likely to report an illness compared with other participants (74/406) (p = 0.048) (Table 7.4). In addition, participants that had ticks attached to them which were positive for either non-pathogenic *Rickettsia* spp. (n=22) or *R. amblyommii* (n=100) were not significantly more likely to become sick as compared to all other participants (number positive/ total: 73/389 and 58/311 respectively) (p=1.00 for both).

Two participants tested positive for RMSF via a blood assay. Of those, one was exhibiting fever and vomiting and reported an IgM titer of 1:64 and an IgG of 0. However, the patient also reported having been previously diagnosed with RMSF via a blood assay, with the date of this diagnosis being unknown. Furthermore, the patient had submitted an *A. americanum* adult which was PCR negative. The second participant reporting RMSF positive results had submitted a single *D. variabilis* adult testing positive for *R. montana*. The patient reported having had no symptoms but had been tested as a pre-cautionary measure by their physician after the tick bite. The patient reported having an IgM of 0 and an IgG of 1:64, with no prior history of RMSF.

Participants who reported an illness were asked if they had been previously diagnosed with a tick-borne disease and eight participants indicated they had a previous diagnosis, of which, three were presumptively diagnosed by a physician (all with Lyme disease) and five were reportedly confirmed with a diagnostic assay (three for RMSF, one for HME, and one for Lyme disease).

Thirty-two participants sought medical attention due to their illness. Nine had blood drawn and 23 were prescribed some type of medication. Interestingly, an additional seven participants went to a physician as either a pre-cautionary measure and/or to have the tick removed, or for an un-related reason and reported being prescribed preventive antibiotics for a tick-borne illness despite a lack of symptoms or laboratory findings. Furthermore, three other participants exhibited no symptoms, but self-medicated with antibiotics.

The final diagnoses of patients whom sought medical attention due to illness were not recorded in this study. However, the data of the 27 participants who reported an illness and had a PCR positive tick attached to them were evaluated to determine if the illness fit the criteria of a potential tick-borne illness. Criteria to be considered a potential tick-borne illness included attached for longer than 6 hours (or length of attachment was unknown), incubation period a minimum of 4 days (to exclude rash development or severe reactions to tick bites which could occur prior to four days), and the individual exhibited at least one of the following symptoms or signs: fever, chills, nausea, rash, stomach pain, weakness, myalgia, appetite loss, dizziness, headache, diarrhea, weight loss, vomiting, and/or sweats but did not have a cough, sore throat, or sinus congestion. Twelve of the 27 individuals evaluated exhibited symptoms that could potentially be

related to a tick-borne illness (Table 7.5). Interestingly, two of the individuals with *R. amblyommii*-positive ticks whom reported symptoms conducive with a potential tick-borne illness also reported a bulls-eye rash at the tick bite site. However, in one case, multiple ticks were submitted by the participant and it could not be determined whether the *R. amblyommii*-positive tick was located at the tick bite site around which the rash occurred.

In the univariate analysis, time of tick attachment, use of secondary protection, and presence of pathogens were all significantly associated with illness (Table 7.6). Upon performing a multivariate analysis with logistic regression, time attached was found to be the only variable significantly associated with development of illness ($p = 0.037$). Compared to patients that had ticks attached for less than 12 hours, those with ticks attached for 12-24 hours (OR [95%CI] = 2.0 [1.0, 4.0]) and for 24-48 hours (2.9 [1.3, 6.5]) were significantly more likely to become sick, while those with ticks attached for > 48 hours (1.5 [0.6, 3.9]) were not significantly more likely to report an illness.

Finally, data were collected regarding interactions with domestic animals. Four hundred and twelve participants owned dogs and 73 owned cats. One hundred and sixty-four dog owners and 15 cat owners allowed their dog/cat in the home. When asked if anti-tick medication had been used on their pets in the past 30 days, 27 cat owners (10 of which were allowed in the home) and 162 dog owners (126 of whom allowed their dog/s in the house) replied yes. It was also found that 42 ($n = 412$) worked with animals. Presence of dogs and/or cats in the home, use of anti-tick medication, and regular interaction with work animals, was not correlated with type of tick species or pathogen prevalence.

Discussion

This study was one of the most comprehensive epidemiologic studies to date on human tick bites. In addition to providing information related to tick distribution around the state, it provided a rare opportunity to follow individuals bitten by bacteria positive ticks to determine whether a potential tick-borne illness developed. Furthermore, it provided useful information regarding protective measures used by the general public and risk factors for illness which could be used to better target and better educate the general public on tick-borne disease prevention.

This study collected valuable information related to distribution of tick species at a county level throughout Georgia and in particular provides new county-level data on *A. maculatum* distribution throughout the state. It is of interest that prevalence of non-pathogenic *Rickettsia* was significantly higher in the Coastal region, which was not associated with the distribution of its primary vector, *A. americanum* which was more common in the Piedmont region. This trend potentially indicates that non-pathogenic bacteria has a higher prevalence in the Coastal region of Georgia.

Individuals with ticks attached to them for greater than 12 hours were more likely to develop illness, which was not surprising because most tick-borne pathogens require 12-24 hours (and in some cases longer) to be transmitted (Azad and Beard 1998, Parola et al. 2005, Hynote et al. 2012). However, because illness was self-reported by the participants, we cannot confirm the causative agents responsible for the illnesses. Furthermore, the findings that some individuals either self-medicated and/or were put on antibiotics by a physician despite laboratory and/or clinical symptoms are concerning on a number of levels. These actions allow these individuals to unnecessarily take antibiotics

and particularly in the case of self-medication, could result in the individual taking the wrong type and/or dosage of medication. Furthermore, both scenarios result in potential tick-borne diseases not being reported. While it is generally accepted that tick-borne diseases are under-reported, to what degree is unclear (Meek et al. 1996, Lacz et al. 2006, Masters et al. 2008). Overall, our study found that in individuals bitten by a tick, 2.7% (n = 12) developed a possible tick-borne disease. Interestingly, there were only two instances in our study in which it was reported by the participant that their physician reported their case as a tick-borne disease to the state health department, neither of which fit our criteria for a potential tick-borne disease. Although our study relied on self-reported illness without definitive diagnoses and was biased due to the fact that participants a) found the ticks attached to them (versus having not), and b) perhaps had better overall awareness of tick-borne diseases as evidenced by them participating in the study, it still provides insight in to the possible extent of misdiagnosis and under-reporting of tick-borne diseases.

In addition to gaining insight into under- and mis-diagnosis of tick-borne diseases, this prospective study also allowed us to follow individuals bitten by ticks positive for bacteria that has more recently been questioned as being potentially pathogenic such as *Borrelia lonestari* (originally suspected as a causative agent of STARI) (James et al. 2001, Masters et al. 2008) and *Rickettsia amblyommii* (recently questioned as causing rickettsiosis in some individuals) (Apperson et al. 2008). While *B. lonestari* was not associated with any of the individuals fitting the criteria for a potential tick-borne disease, eight of our 12 possible cases were associated with *R. amblyommii,* two of which were associated with an erythema migrans. Similarly, Billeter et al. (2007) reported a single

case in which *R. amblyommii* was directly associated with a tick bite resulting in a rash. Upon administering doxycycline, the rash dissipated and no other clinical symptoms were reported. These observations appear to indicate that further research is warranted regarding the potential pathogenicity of *R. amblyommii*.

Prevention measures are critical to decrease the risk of tick infestation and agencies have numerous recommendations for preventing exposure (Stafford 2004, Torres and Carey 2004, Centers for Disease Control and Prevention 2012). We noted that many individuals included in the study used primary or secondary preventative measures to avoid infestation with ticks. Unfortunately, all participants had ticks attached so the measures were not 100% effective, but it is not possible for us to evaluate the effectiveness of these measures because we did not have control individuals. However, these data do indicate that only a small percentage (34%) of the general public being bitten by ticks is purposely utilizing tick bite prevention measures. Interestingly, there was no bias among gender among those individuals using preventive measures, and although there was low racial diversity, it appears that current public education initiatives against tick bites are reaching a variety of demographics. However, because few people knowingly utilized prevention measures, additional outreach programs are needed.

It was also found that sites of body attachment were significantly associated with tick species. While our findings indicate that full body tick checks performed immediately after tick exposure may be the best way to prevent tick-borne illness, it is equally crucial to perform effective body checks. Our findings corroborate the findings of Felz and Durden (1999) whom also found that *D. variabilis* preferred attachment sites on the head, while *A. americanum* preferred the trunk and extremities. Knowing which parts

of the body different tick species tend to attach to in conjunction with tick distribution and activity periods may allow for more effective tick checks.

In conclusion, several important findings are reported in this study related to the epidemiology of human tick-infestation in Georgia, tick bite prevention, and risk of human tick-borne pathogens. Identifying individuals fitting criteria for a possible tick-borne illness while finding others whose physicians were potentially incorrectly reporting a tick-borne disease provides evidence towards the common assumption that tick-borne diseases are misdiagnosed and underreported. Furthermore, our data provide valuable reports of tick species occurrence and seasonality in Georgia. Collectively, these observations in conjunction with our findings regarding tick attachment site preferences by tick species and personal protective measures used against tick bites provide information that can be used for better public and physician education on tick bite prevention and tick-borne disease diagnosis. Finally, due to study design, correlations between illness and the specific ticks tested in this study are speculative, so more detailed follow-up studies are recommended to further investigate the risk of developing a tick-borne disease following infestation with both pathogen-positive ticks as well as *R. amblyommii* positive ticks.

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Table 7.1. Pathogens tested for and PCR protocols used for all ticks collected in this study.

Pathogen	Gene Target / Reference	Primers	Thermal Cycling Conditions	
			Primary	Secondary
E. chaffeensis	16S rRNA / Dawson et al., 1994	Primary: ECC (5'-AGAACGAACGCTGGCGGCAAGCC) Primary: ECB (5'-CGTATTACCGCGGCTGCTGGCA) Secondary: HE1 (5'-CAATTGCTTATAACCTTTTGGTTATAAAT) Secondary: HE3 (5'-TATAGGTACCGTCATTATCTTCCCTAT)	1. 94°C for 1:30 2. 94°C for 0:25 3. 55°C for 0:15 4. 72°C for 0:30 5. cycle for 34x 6. 72°C for 5:00	1. 94°C for 1:00 2. 55°C for 1:00 3. 72°C for :15 4. cycle for 39x
E. ewingii	16S rRNA / Dawson et al., 1994; Dawson et al., 1996	Primary: ECC (5'-AGAACGAACGCTGGCGGCAAGCC) Primary: ECB (5'-CGTATTACCGCGGCTGCTGGCA-3') Secondary: HE3 (5'-TATAGGTACCGTCATTATCTTCCCTAT-3') Secondary: EE72 (5'-CAATTCCTAAATAGTCTCTGACTATT-3')	1. 94°C for 1:30 2. 94°C for 0:25 3. 55°C for 0:15 4. 72°C for 0:30 5. cycle for 34x 6. 72°C for 5:00	1. 94°C for 3:00 2. 95°C for 0:15 3. 48°C for 0:30 4. 72°C for 0:30 5. cycle for 40x 6. 72°C for 7:00
Rickettsia spp.	17-kDa antigen / Webb et al., 1990; Labruna et al., 2004	Primary: 17kD1 (5'-GCTCTTGCAACTTCTATGTT-3') Primary: 17kD2 (5'-CATTGTTCGTCAGGTTGGCG-3') Secondary: 17k-5 (5'-GCTTTACAAAATTCTAAAAACCATATA) Secondary: 17k-3 (5'-TGTCTATCAATTCACAACTTGCC)	1. 95°C for 3:00 2. 95°C for 0:15 3. 48°C for 0:30 4. 72°C for 0:30 5. cycle for 40x 6. 72°C for 7:00	1. 95°C for 3:00 2. 95°C for 0:15 3. 48°C for 0:30 4. 72°C for 0:30 5. cycle for 40x 6. 72°C for 7:00

Borrelia spp.	Flagellin (*flaB*) / Barbour et al., 1996	Primary: FLALL (5'-ACATATTCAGATGCAGACAGAGGT) Primary: FLARL (5'-GCAATCATAGCCATTGCAGATTGT) Secondary: FLALS (5'-AACAGCTGAAGAGCTTGGAATG) Secondary: FLARS (5'-CTTTGATCACTTATCATTCTAATAGC)	1. 95°C for 3:00 2. 95°C for 1:00 3. 55°C for 1:00 4. 75°C for 1:00 5. cycle for 40x	1. 95°C for 3:00 2. 95°C for 1:00 3. 55°C for 1:00 4. 75°C for 1:00 5. cycle for 40x
F. tularensis	FopA / Fulop et al., 1996	Primary: FNA8L (5'-CGAGGAGTCTCAATGTACTAAGGTTTGCCC) Primary: FNB2L (5'-CACCATTATCCTGGATATTACCAGTGTCAT) Secondary: FNA7L (5'-CTTGAGTCTTATGTTTCGGCATGTGAATAG) Secondary: FNB1L (5'-CCAACTAATTGGTTGTACTGTACAGCGAAG)	1. 94°C for 3:00 2. 94°C for 0:15 3. 55°C for 0:15 4. 72°C for 0:30 5. cycle for 34x 6. 72°C for 5:00	1. 94°C for 3:00 2. 94°C for 0:15 3. 55°C for 0:15 4. 72°C for 0:30 5. cycle for 34x 6. 72°C for 5:00

Table 7.2. Associations between primary and secondary protective measures against tick bites and development of illness, gender, and proximity to one's home residence.

	Illness After Tick Bite (%)			Gender (%)			Location When Bitten (%)			
	Sick	Not Sick	p-value (protection vs. illness)	Male	Female	p-value (gender vs. protection)	Away from Home	At Home	Unknown	p-value (location vs. protection)
No Primary Protection* (n=14)	5 (36)	9 (64)		9 (64)	5 (36)		2 (14)	11 (78)	1 (8)	
Primary Protection (n=55)**	11 (20)	44 (80)	0.29	27 (49)	28 (51)	0.52	27 (49)	19 (35)	9 (16)	0.18
No Secondary Protection* (n=10)	5 (50)	5 (50)		4 (40)	6 (60)		5 (50)	5 (50)	0	
Secondary Protection (n=59)**	11 (19)	48 (81)	0.04	32 (54)	27 (46)	0.19	24 (41)	25 (42)	10 (17)	0.4

*5 participants not using primary or secondary protection did not answer questions related to illness, gender or location when bitten on purpose

**Note that 12 of 55 and 25 of 59 individuals took primary and secondary protection measures respectively.

Table 7.3. All ticks and pathogens identified in each tick species.

Tick Species	Total Collected	Sex		Life Stage		Organisms Detected
		Male	Female	Nymph	Adult	
*A. americanum**	426/124	112/31	129/47*	185/46	241/78	1 *E. chaffeensis* 2 *B. lonestari* 122 *Rickettsia* spp. 117 *R. amblyommii* 1 *R.* sp. TR-39 1 *R. rickettsii* 1 *R. rhipicephali* 1 *R. montana* 1 *R.*sp.
D. variabilis	142/20	57/9*	85/11	0	142/20	2 *E. ewingii* 1 Panola Mountain Ehrlichia species 18 *Rickettsia* spp. 15 *R. montana* 2 *R. amblyommii* 1 *R.* sp. ARANHA
I. scapularis	7/6	0	7/6	0	7/6	6 *Rickettsia* spp. 4 *R.* sp. TR-39 1 *R.* sp. Is-1 1 *R. cooleyi*
A. maculatum	19/5	9/1	9/2	1/1	19/3	5 *Rickettsia* spp. 1 *R. parkeri* 2 *R. amblyommii* 1 *R.* sp ARANHA 1 *R.* sp.

Amblyomma sp.	3/2	0	0	3/2	0	*2 R. amblyommii*
TOTAL	603/157	32	60	44	101	

Table 7.4. All individuals having submitted a tick positive for pathogenic bacteria.

Patient #	Tick Species	Pathogen	Time Attached (hrs)	Incubation, if applicable (days)	Went to Doctor	Symptoms
1	*D. variabilis*	*E. ewingii*	unknown	4	yes	fever, rash, weakness, myalgia, joint pain, congested, earache, swollen & runny eyes
2	*D. variabilis*	*E. ewingii* (& *R. montana*)	6-12	<1	yes	tight neck
3	*D. variabilis*	Panola Mountain *Ehrlichia*	2-6	n/a	no	not sick
4	*A. americanum*	*E. chaffeensis (& R. amblyommi*)	1-4	13	no	nausea, fever, chills, stomach pain, weakness, headache, joint pain, sweats, cough
5	*A. maculatum*	*R. parkeri*	unknown	n/a	no	not sick

Table 7.5. All participants meeting criteria for exhibiting a potential tick-borne illness.

Patient #	Time Attached (hrs)	Incubation (days)	Days Sick	First Symptom/s	Symptoms	Rash at Site of Bite	Rash Description	Went to Doctor
2	6-12	0	2	tight neck	tight neck	n/a		yes
6	unknown	6	unknown	lump in neck	joint pain	n/a		no
7	6-12	12	1	fatigue	chills, nausea, stomach pain, weakness, muscles, loss of appetite, dizziness, headache, joint pain	n/a		no
8	>48	11	2	diarrhea	stomach pain, weakness, diarrhea	n/a		no
9	unknown	unknown	14	body aches & fever	fever, rash, weakness, myalgia, dizziness, headache, joint pain	yes	bull's-eye; grew to 13-15 cm diameter	yes
10	24-48	5	21	fatigue	fever, nausea, rash, weakness, myalgia, dizziness, headache	yes	raised, reddish, swollen	yes
11	>48	17	6	headache	nausea,	n/a		no

					weakness, loss of appetite, headache			
12	24-48	1	14	rash	rash, joint pain	yes	red, 3 cm diameter.	yes
13	24-48	5	7	insomnia	chills, stomach pain, weakness, loss of appetite, headache, joint pain, sweats	n/a		yes
14	12-24	26	2	fever	fever, chills, nausea, stomach pain, weakness, myalgia, loss of appetite, sweats	n/a		no
15	unknown	15	unknown	pain in neck	pain in neck	n/a		no
16	24-48	4	unknown	sore eyes, extreme fatigue, fever	fever, nausea, rash, weakness, myalgia, photosensitivy, headache, diarrhea, joint pain, confusion	yes	bull's-eye	no

Table 7.5 cont. All participants meeting criteria for exhibiting a potential tick-borne illness.

Patient #	Medicine Prescribed (if any)	Species	Stage	Engorged	Pathogen
2	biaxin	*D.variabilis*	adult	no	*E. ewingii* & *R. montana*
6		*D. variabilis*	adult	partially	*R. montana*
7		*A. americanum*	adult	no	*R. rhipicephali*
8		*I. scapularis*	adult	partially	*Rickettsia* sp. TR-39
9	doxycyline	7 *A. americanum*	nymphs	no	1 *R. amblyommii*
10	doxycycline	7 *A. americanum*	nymphs	no	4 *R. amblyommii*
11		2 *A. americanum*	adult & nymph	no	1 *R. amblyommii*
12	unknown topical cream	2 *A. americanum*	nymphs	no	1 *R. amblyommii*
13	tetracycline	*Amblyomma* sp.	unknown	unknown	R. amblyommii
14		*D.variabilis*	adult	partially	*R. amblyommii*
15		*A. americanum*	adult	no	*R. amblyommii*
16		*A. americanum*	adult	no	*R. amblyommii*

Table 7.6. Univariate analysis of potential risk factors for illness after a tick bite. Participants with missing information were excluded from statistical comparisons.

Variable	n	# Sick	*P
Time Attached (hrs)			0.032
1-12	196	26	
12-24	68	16	
24	39	12	
>48	33	6	
Secondary Protection			0.045
yes	59	11	
no	10	5	
Pathogen Positive			0.048
yes	5	3	
no	406	74	

*Exact test of independence

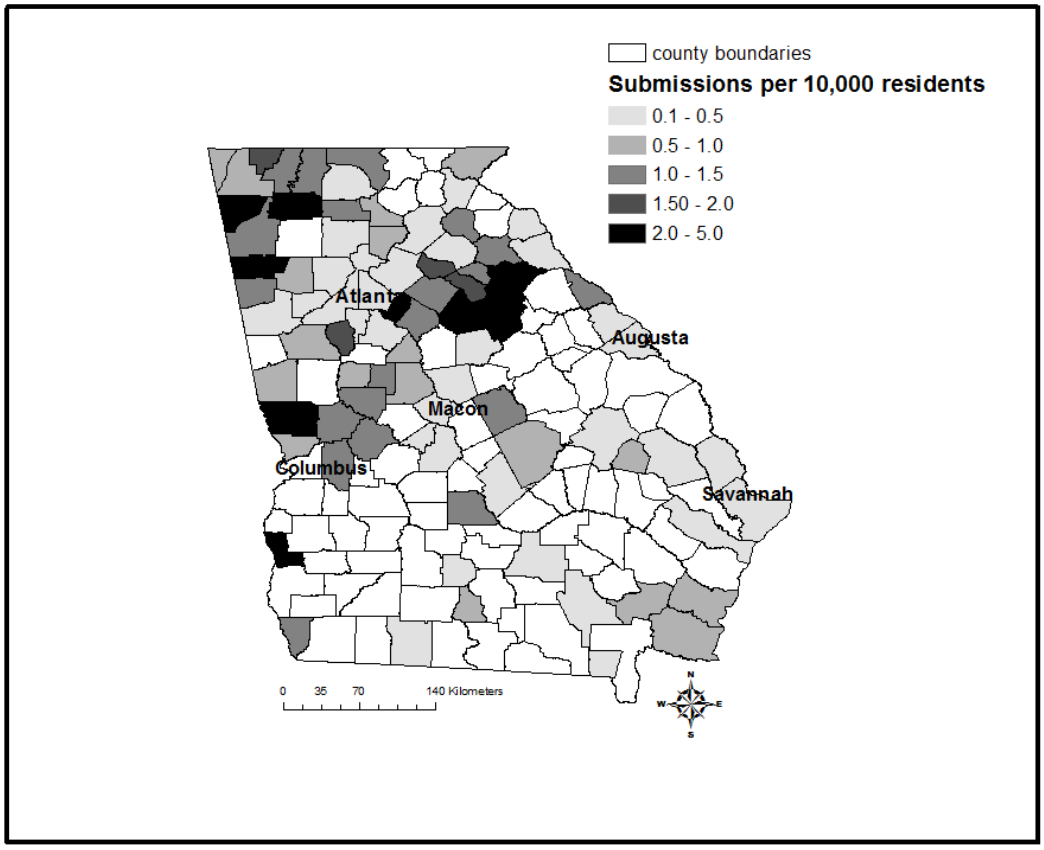

Figure 7.1. Georgia state map of total submissions per 10,000 residents by county. Note that map denotes participants' resident county, not necessarily where the tick originated.

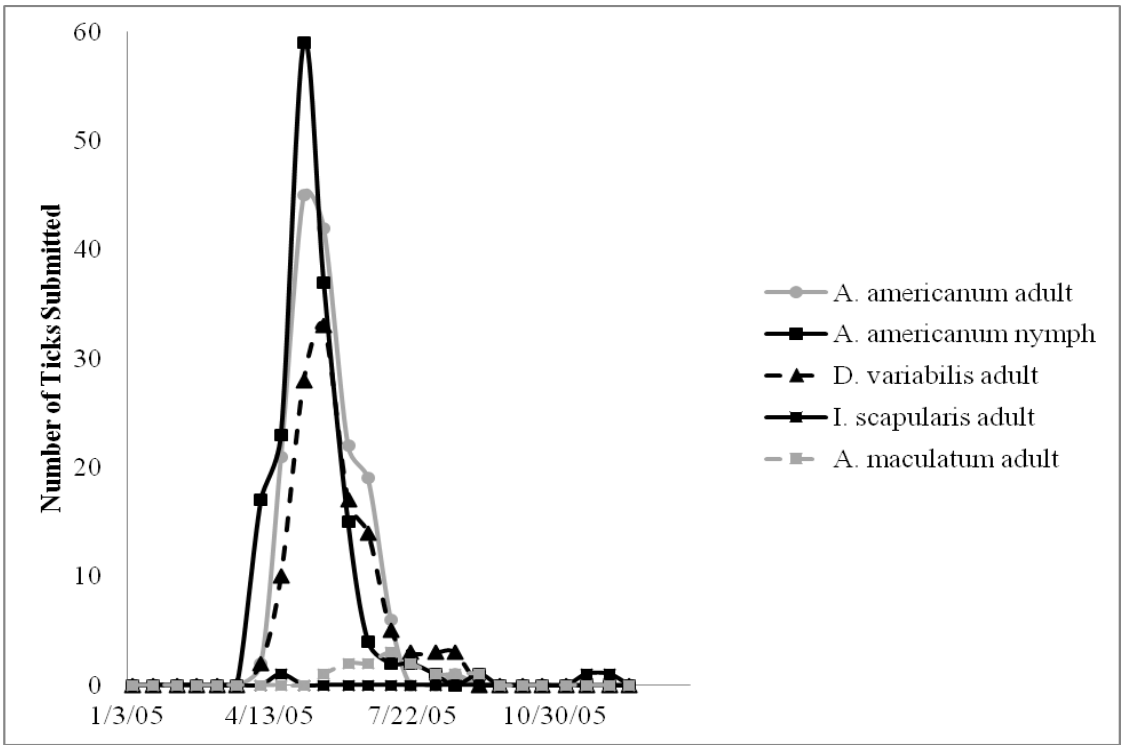

Figure 7.2. Seasonality of tick submissions in 2005. Note that tick submissions were too low in 2006 to warrant graphing.

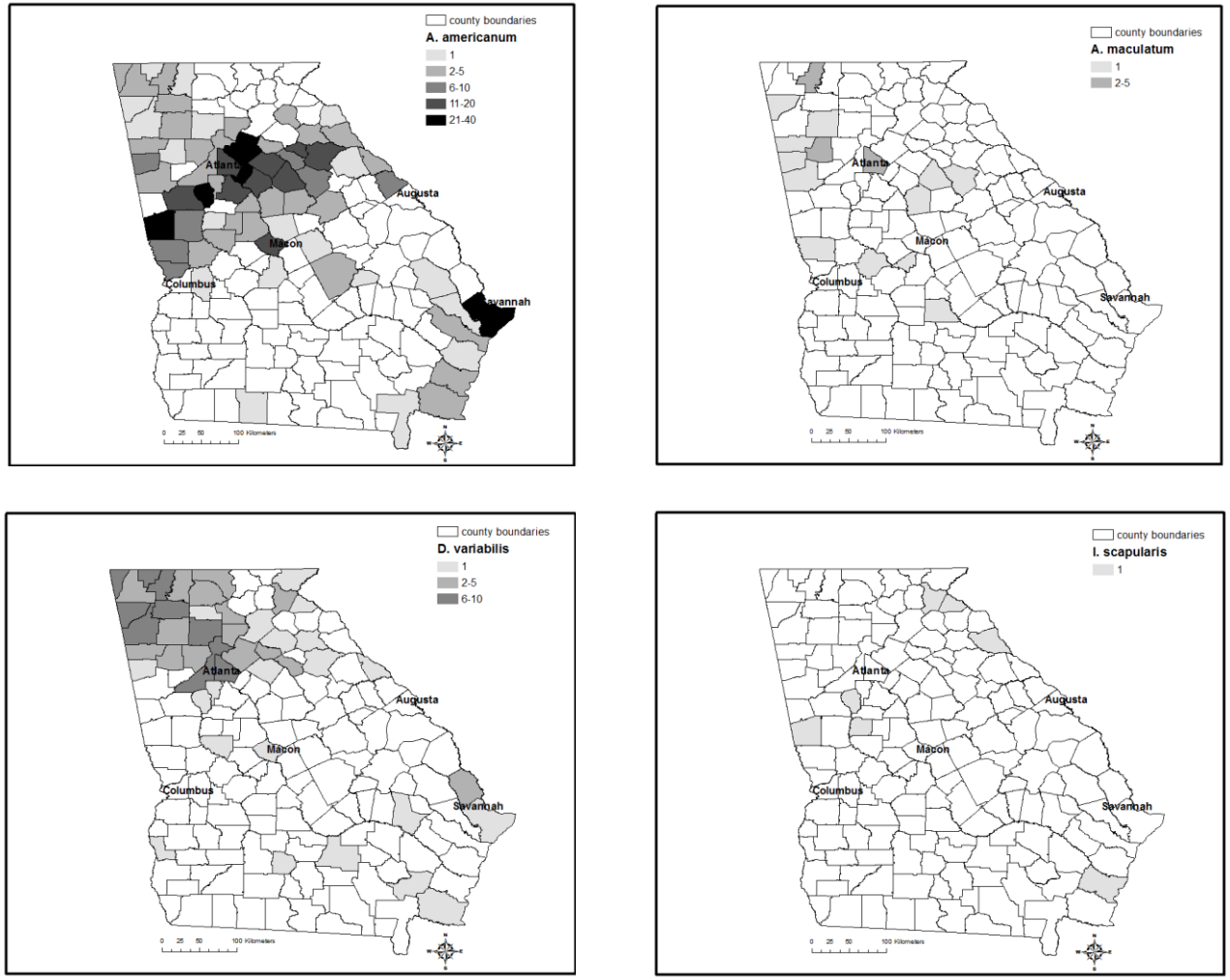

Figure 7.3. Maps of numbers of ticks originating from each Georgia county by tick species.

CHAPTER 8

CONCLUSIONS

The ultimate goal of this dissertation was to determine the impacts of long-term prescribed burning on ticks and tick-borne pathogen prevalence while also better elucidating tick phenology in Georgia. As tick-borne pathogen incidence and emergence increase, the importance of having an understanding of tick population dynamics unique to each georegion while identifying effective tick control methods are especially prudent.

Study 1 (Chapter 3)

In contrast to a number of studies which have seen only temporary or no reductions in ticks after prescribed burning (Hoch et al., 1972; Davidson et al., 1994; Cilek and Olson, 2000, Willis et al. 2012), our study found that prescribed fire, when performed on a long-term basis, significantly and permanently reduced tick populations regardless of other variables. Furthermore, a shift in species composition was observed with *A. americanum* preferring unburned sites, surrounded by unburned sites (UBUB) and *A. maculatum* preferring burned sites, surrounded by burned sites (BB). To our knowledge, this is the first time such a trend has been observed and has implications for public health as *A. americanum* transmits the causative agents of human monocytic ehrlichiosis (HME), ehrlichiosis *ewingii*, Panola Mountain *ehrlichiosis*, and Southern tick-associated rash illness (STARI) whereas, *A. maculatum* transmits the causative agent

of American boutonneuse fever. Regardless of the species composition, the significantly lower counts of ticks occurring in burned habitat should ultimately lower the density of infected ticks, however pathogen testing of ticks collected is needed to gain a complete understanding of disease risk. It is hypothesized that the significant and apparently permanent reduction of ticks observed in this study are due to the long history of prescribed fire which over time has altered habitat conditions leading to a dry, hot microclimate in which few ticks can survive. Thus, prescribed fire is a promising tool for tick control and its applicability in different management and landscape conditions should be investigated.

Study 2 (Chapter 4)

Ehrlichia chaffeensis (0.2%), *E. ewingii* (0.7%), Panola Mountain *Ehrlichia* (0.2%) in *Amblyomma americanum*, and *Anaplasma phagocytophilum* (1.0%) in *Ixodes scapularis* were all found in prevalences similar to what has been observed in previous studies of the surrounding regions (Clark 2004, Mixson et al. 2006, Cohen et al. 2010). *R. parkeri* was detected at a minimum prevalence of 1.4% in *A. maculatum* (Sumner et al. 2007, Fornadel et al. 2009), however, this relativel low prevalence is most likely due to the fact that we were unable to identify a number of *Rickettsia* spp. detected in these ticks. Interestingly, *A. americanum* were found to have significantly lower prevalences of *Rickettsia* spp. in burned sites as compared to unburned sites. All *Rickettsia* spp. in *A. americanum* are non-pathogenic endosymbionts, however, the potential pathogenicity of *R. amblyommii* has been questioned in more recent years (Billeter et al. 2007). Of note was the absence of *Borrelia* spp. and the low prevalence of *A. phagocytophilum* in *I.*

scapularis. In recent years, there has been great interest surrounding the fact that human granulocytic anaplasmosis (*A. phagocytophilum*) and particularly Lyme disease (*B. burgdorferi*) have such exceptionally low incidences in the Southeast despite the presence of its vector, *I. scapularis* (Rosen et al. 2012, Stromdahl and Hickling 2012). Although the cause behind this trend is not entirely understood, our findings seem to corroborate the general trend of low prevalences of these pathogens in the south (Fang et al. 2002, Yabsley et al. 2009). Overall, prescribed fire does not significantly reduce the prevalence of known pathogenic tick-borne bacteria, however, when accounting for reductions in tick counts in burned areas, it was found that the density of infected ticks was nearly 35 times lower in burned areas versus unburned areas. This indicates that burning does reduce risk of disease and the applicability of this tick control method should be investigated in other management scenarios.

Study 3 (Chapter 5)

Several observational and experimental studies performed in the past have documented *Solenopsis invicta* (red imported fire ants [RIFA]) predating and/or reducing tick populations (Harris and Burns 1972, Burns and Melancon 1977, Fleetwood et al. 1984), particularly *A. americanum*. While the impacts of RIFA on *A. americanum* could not be determined due to our study design, RIFA was not found to have a significant effect on *A. maculatum*. This is most likely due to the fact that *A. maculatum* and RIFA peak activity do not overlap (Porter and Tschinkel 1987, Cilek and Olson 2000, Gleim et al. unpublished data), resulting in the two species not interacting.

Interestingly, burned habitat was found to have a significant impact on *A. americanum* survival with *A. americanum* survival being significantly higher in unburned sites as compared to burned sites and within the burn sites, having significantly lower survival than *A. maculatum*. It is hypothesized that this is due to physiologic and/or behavioral traits possessed by *A. maculatum* that allow it to survive more successfully in hot, dry climates (as is experienced in burned areas) as compared to *A. americanum.* Indeed, Needham (1991) found that *A. maculatum* had lower whole-body permeability than *A. americanum*, allowing it to better retain moisture. Thus, while fire has direct effects on tick survival via the burn itself, it appears that the indirect effects of burning (.e.g., changes in vegetation leading to a harsher microclimate) are also significant and perhaps behind the long-term reductions of ticks and shift in species composition observed in Study 1.

Study 4 (Chapter 6)

Amblyomma tuberculatum is a unique tick species restricted to the distribution of its primary host, the gopher tortoise (*Gopherus polyphemus*) (Bishop and Trembley 1945). Gopher tortoises are typically associated with long-leaf pine ecosystems (fire-dependent ecosystems) and in particular prefer burned habitats for its lush and diverse understory forbs, open mid-story and semi-open canopy (Mitchell 2005, Smith et al. 2006). *A. tuberculatum* typically feed on their wildlife hosts (rodents, birds, and gopher tortoises) (Bishop and Trembley 1945, Cooney and Hays 1972), however, there have been rare reports of them feeding on humans (Goddard 2002, Gleim unpublished data). In this study, a novel Spotted Fever Group (SFG) *Rickettsia* sp. was identified and

characterized from *A. tuberculatum* larvae. Its high prevalence (50-100%), apparent wide-spread geographic distribution (GA, FL, and MS), and close relationship to several pathogenic SFG *Rickettsia* (*R. parkeri, R. conorii, and R. africae*) indicate the need for further study of this bacteria to determine pathogenicity both to humans and the federally threatened/ endangered gopher tortoise.

Study 5 (Chapter 7)

This study provided valuable insight into tick species occurrence throughout Georgia while providing a unique opportunity to follow possible disease development after being bitten by ticks positive for pathogenic, non-pathogenic, and questionably pathogenic bacteria. Although studies have been performed in the past on ticks parasitizing humans, few have been as large-scale and comprehensive (Harrison et al. 1997, Merten and Durden 2000, Stromdahl et al. 2001, Goddard 2002). It was ultimately found that time of tick attachment, use of secondary protection, and being bitten by a tick positive for pathogenic bacteria were significant predicators of illness. Furthermore, it was found that *D. variabilis* and *A. americanum* are significantly more likely to attach to certain areas of the body. These findings in conjunction with the information obtained regarding tick phenology of Georgia provides valuable insight that could be used to improve the general public and physicians' understanding on tick-borne disease prevention.

The finding that 12 participants fit the criteria of a possible tick-borne disease was of particular interest, particularly in light of the fact that eight of those cases (two of which were associated with a rash at the tick bite site) were associated with *R.*

amblyommii, a bacteria who's potential for pathogenicity has more recently been questioned (Billeter et al. 2007). Although this study was restricted by participant self-reporting and the inability to obtain confirmed diagnoses from physicians, this was still a valuable exercise that has provided important insight in to potential misdiagnosis and under reporting of tick-borne diseases.

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