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Adjacent habitat distribution and management of the lone star tick in Tennessee

Christopher Glenn Morris

To the Graduate Council:

I am submitting herewith a thesis written by Christopher Glenn Morris entitled "Adjacent habitat distribution and management of the lone star tick in Tennessee." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Reid R. Gerhardt, Major Professor

We have read this thesis and recommend its acceptance:

Jerome Grant, Craig Reinemeyer, Arnold Saxton

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

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Dr. Reid R. Gerhardt, Major Professor

We have read this thesis
and recommend its acceptance:

Arnold M Saxton

Accepted for the Council:

Associate Vice Chancellor and
Dean of the Graduate School

ADJACENT HABITAT DISTRIBUTION AND MANAGEMENT OF THE LONE STAR TICK IN TENNESSEE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Christopher Glenn Morris

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ABSTRACT

A six-year treatment program involving the administration of ivermectin to white-tailed deer to manage a population of lone star ticks (LST) implicated in the transmission of *Ehrlichia chaffeensis* Anderson, Dawson, Jones & Wilson to humans in 1993 was performed in Fairfield Glade, TN, on the Cumberland Plateau from 1994 to 1999. *Ehrlichia chaffeensis* is the causative agent of human monocytic ehrlichiosis (HME). Two concentrations of ivermectin, 250 mg and 500 mg / 22.7 kg whole kernel corn, were administered from 1994 to 1996 and 1997 to 1999, respectively. Treatment was typically initiated in March and ended in July of each year in compliance with Food and Drug Administration guidelines.

All free-living LST stages were sampled in the first treatment area (FTA) and non-treatment area (NonTA) from 1994 to 1999. These sites and an additional treatment area (NTA) were sampled from 1997 to 1999. In each of the three areas, three sites were selected, and wooded and open, grassy areas were sampled at each site. Cloth flags (1 m x 1 m were used to sample all free-living LST stages. In addition CO_2 traps (1 m x 1 m) were used to sample adult and nymphal LSTs.

Reductions in all stages of the LST population were observed in the FTA from 1994 to 1996 and the NTA from 1997 to 1999 relative to the NonTA. The effect of the two concentrations of ivermectin was equivalent on male and female LSTs. Natural

fluctuations in the LST population and absence of 1999 larval mass data at the time of this publication make this estimate for LST nymphs and larval masses inconclusive.

Comparison of two sampling methods revealed that CO_2 traps placed in wooded habitats during spring captured LST males and females more frequently. Nymphal LSTs were captured more frequently by cloth flags in wooded habitats during summer and early fall. No comparison was possible for larval masses of LSTs, because CO_2 traps were not used to sample them.

Adjacent habitats of woods, ecotone and open, grassy areas were sampled to determine the most likely area one would encounter LSTs. Three sites were selected on the golf course that was the focus of the 1993 HME epidemic and three additional roadside sites were selected in the same area. Lone star tick larval masses were most abundant 5 and 10 m into the woods and nymphs were most abundant 10 m into the woods. No differences were found for LST nymphs and larval masses between golf and roadside sites. Few adults were captured on the six sites which prevented thorough statistical analysis. Fisher's exact test (FET) suggested that males were most abundant in the ecotone of both golf and roadside sites. Females appeared most abundant in the ecotone and 10 m into the woods on golf course sites, but in roadside sites they were most abundant 10 m into the woods.

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CHAPTER I

INTRODUCTION

i. Lone Star Tick Biology

The lone star tick (LST), *Amblyomma americanum* (L.), is regarded as a severe pest to humans and domestic animals. It is generally considered to be restricted to the southeastern and south-central United States, but recent reports place it as far north as New York and Maine (Means & White 1997, Keirans & Lacombe 1998). Lone star tick problems have been particularly severe for farmers in Oklahoma where it is not uncommon for a cow to be parasitized by several thousand ticks at once (Hair & Howell 1970). Wounds from the bites of LSTs served as a point of entry for the primary screwworm (Bishopp & Trembley 1945, Cooley & Kohls 1944) before the success of the screwworm eradication program. Lone star ticks also have been responsible for detriment to wildlife fauna, such as white-tailed deer, *Odocoileus virginianus* (Zimmerman), by means of tissue destruction (Bolte et al. 1970). In 1968, about 34% of fawns on a 5,700 hectare refuge perished as a result of LST tissue destruction (Bolte et al. 1970). In addition to damage caused by the ticks themselves, they have been implicated as vectors of a wide array of microbial pathogens.

Lone star ticks thrive in areas where large ruminant populations are well established. Direct observation and quantitative analysis of LST numbers on wild vertebrates reveal the white-tailed deer as the primary host of the LST in forested

habitats, but it has been found on more than 45 species of vertebrates in the United States (Bishopp & Trembley 1945, Hair & Howell 1970). Bishopp & Trembley (1945) also note that more than 4,800 LSTs were removed from a single ear of a captured deer in their study. Woodlands and wooded pastures, where the tree canopy and dense leaf litter prevent desiccation, frequently have large LST populations (Hair & Howell 1970, Semtner et al. 1971b). Open, grassy areas have fewer LSTs than wooded areas, particularly in geographic regions with lower humidities and higher temperatures (Hair & Howell 1970, Semtner et al. 1971a, 1971b, Semtner & Hair 1973a, 1973b).

The life cycle of the LST is the typical 3-host pattern seen in several ixodid species (Hair & Howell 1970, Sonenshine & Levy 1971). Lone star tick behavior and the level of seasonal activity vary depending on geographic region (Semtner & Hair 1973b). Lone star ticks are active throughout the year in Texas and Oklahoma, although only a few ticks can be found during winter months (Drummond 1967, Hair & Howell 1970, Semtner & Hair 1973a). Lone star ticks in the southeastern United States typically have a more narrow seasonal range of March to late October with no ticks observed from late November through December (Lancaster 1955, Davidson et al. 1994a, Jackson et al. 1996, Gerhardt et al. 1999).

Although considerable geographic variation in seasonal activity exists, several authors describe a pattern similar to that of Hair & Howell (1970). Several thousand larvae emerge from an egg mass between late July and October. Larvae successful in acquiring hosts engorge with blood upon attachment and drop off after 3 to 5 days. They molt to nymphs 1 to 3 weeks after detachment although field observations have been

made of engorged larvae overwintering before molting to nymphs. The nymphs become active the following March or April. They attach to the host for 4 to 8 days and require up to one month to molt to adults. Adults generally do not actively hunt hosts until the following March or April. They also require 4 to 8 days of attachment to complete feeding.

Lone star tick larvae congregate on low-lying vegetation and form a mass. All larvae in the mass become active upon direct contact with a vertebrate host and attempt to attach. Lone star tick nymphs and adults use the same ambush strategy as the larvae, but they also can actively pursue a host. Generally, they will climb onto various medium height vegetation, such as tall grass or small bushes, and descend when environmental conditions become unsuitable (Hair & Howell 1970, Semtner & Hair 1973a). While on the vegetation, they extend their forelegs perpendicular to their body and become inanimate which is known as questing. While questing, the ticks use various sensilla on their palps and tarsi to sense the presence of a potential host (Sonenshine 1993a).

On the dorsal surface of the tarsi of leg I of all ixodid ticks is Haller's organ which possesses sensilla responsible for sensing heat, moisture and chemicals in the environment (Sonenshine 1993a). Foelix & Axtell (1972) contend that, for the LST, Haller's organ has two functions: olfaction and humidity reception. Among the chemicals relative to hunting that the LST can sense with the sensilla on this organ is carbon dioxide (CO_2). Monitoring CO_2 concentrations in the atmosphere serves as a hunting tool because it allows them to sense the presence of a potential vertebrate host exhaling nearby. When a nymph or adult senses CO_2, it will often descend from questing and

move rapidly toward the source. Positive response of larvae to CO_2 bait trapping has been observed, but their extremely small size generally makes ambush, as opposed to chasing down a host, a more favorable means of host capture (Wilson et al. 1972). However, Kinzer et al. (1990) reported LST larvae were captured with a CO_2 lure when operated for six hours.

Mating typically occurs on the host although anecdotal evidence demonstrates rare mating off the host (Gladney & Drummond 1971). A male takes a bloodmeal, detaches and crawls around the host looking for an attached female. The male mates with a female, drops off the host, and dies. The mated female then engorges to repletion and converts the bloodmeal to several thousand eggs. The female also drops off the host, lays her eggs wherever she lands, and dies (Lancaster 1957). Larval emergence to successful mating and egg laying by adults requires a two-year life cycle (Hair & Howell 1970).

ii. Tick-borne Diseases

The internal physiologies of ticks are closely linked to those of their hosts. This close relationship is evidenced by several enzymes in their saliva including antihemostatic, anti-inflammatory, antiplatelet, antithrombotic, apyrase, bacteriocidal and immunosuppresive agents (Ribeiro et al. 1985, 1991, Alekseev et al. 1995, Kuwabara et al. 1995). These serve to weaken or evade the immune system and encourage steady blood flow which allows the tick to attach to their host, acquire a bloodmeal and detach unnoticed (Ribeiro et al. 1985, Ribeiro 1989). As a result of this physiological intimacy

between a tick and its host, acquisition and transmission of an astonishing array of microbial pathogens are facilitated (Ribeiro 1989, Ribeiro 1990, Alekseev et al. 1995). These pathogens are typically viruses, protozoans, and bacteria (Sonenshine 1993b).

Humans and domestic animals in the United States have been plagued with a wide array of well publicized tick-vectored pathogens over the past century. Since the 1970s, the most prominent of these have been the causative agent of Rocky Mountain spotted fever (RMSF), *Rickettsia rickettsii* (Wolbach), transmitted by the American dog tick, *Dermacentor variabilis* (Say), and the Lyme disease spirochete, *Borrelia burgdorferi* Johnson, Schmidt, Hyde, Steigerwalt, and Brenner, transmitted by the blacklegged tick, *Ixodes scapularis* Say (Burgdorfer 1984). *Ehrlichia* (Rickettsiales: Rickettsiaceae) species, which are in the same family as *R. rickettsii*, have gained notoriety in the past decade with the recognition of the emergence of the human ehrlichioses (Gluckman 1997).

Ehrlichia species are obligate, intracellular, gram-negative coccobacilli (Walker & Dumler 1996, Stayduhar 1997). The importance of these organisms in public and veterinary health has been investigated only recently although they have been recognized as agents of veterinary disease since the discovery of canine ehrlichiosis in 1935 (Walker & Dumler 1996, Gluckman 1997). The first extensive study of the group occurred after 1970 when an epizootic of canine ehrlichiosis killed almost 300 military dogs during the Vietnam war (Robinson & Garner 1973). With the recognition of the agent of the long time serious veterinary problem, Potomac horse fever, as *Ehrlichia risticii* Holland,

Ristic, Cole, Johnson, Baker, and Guetz in 1984 (Holland et al. 1984), the amount of research on the ehrlichiae increased dramatically (Walker & Dumler 1996).

In 1986, the first case of human ehrlichiosis was erroneously diagnosed as infection of *E. canis* (Donatien and Lestoquard) (Maeda et al. 1987, Goddard 1997). In 1991, a soldier at Fort Chaffee, Arkansas, was the first person to be officially diagnosed with human ehrlichiosis with the causative agent described as a unique pathogen similar to *E. canis* (Dawson et al. 1991). The new species was named *E. chaffeensis* Anderson, Dawson, Jones & Wilson after the fort. This disease is focused primarily in the southeastern United States with Maryland as the current northern boundary (Centers for Disease Control 1994). *Ehrlichia chaffeensis* attacks the monocytes of its host, which led to the recognition of a second emerging human ehrlichiosis when physicians observed attacks on the granulocytes of 12 patients by an unknown ehrlichial pathogen in Wisconsin and Minnesota (Bakken et al. 1994). This currently unidentified *E. equi*-like organism and *E. chaffeensis* have consequently been dubbed human granulocytic ehrlichiosis (HGE) and human monocytic ehrlichiosis (HME), respectively (Walker & Dumler 1997, Dumler & Bakken 1998).

Symptoms of the two diseases are almost identical and include fever, headache, myalgia, shaking chills, malaise and other clinical manifestations that may vary from patient to patient (Stayduhar 1997, Walker & Dumler 1997, Dumler & Bakken 1998, Patel & Byrd 1999, Weaver et al. 1999). Fatality rate estimates are currently 2 to 5% for HME and 7 to 10% for HGE (Goddard 1997). Exact diagnosis is confirmed by indirect fluorescent antibody test (IFA), positive polymerase chain reaction (PCR) or growth of

the HME or HGE agents in tissue culture (Anderson et al. 1992, Gluckman 1997, Bakken 1998). However, Radetsky (1998) stresses the need for diagnosis by clinical means so treatment can begin immediately. The current recommendation for treatment in all cases of HME and HGE is doxycycline (Gluckman 1997, Radetsky 1998, Bakken 1998).

iii. Lone Star Tick Management with Ivermectin

Recent implication of LST as the vector of *E. chaffeensis* and other emerging pathogens has increased the need for creative, effective management measures (Anderson et al. 1991, 1992). Success of a management program depends on a wide variety of factors that must be carefully considered. Degree and cost of management, potential social resistance, and potential impact of management measures on the environment must be determined for each new management method before it can be used on a regular basis (Sonenshine 1993b).

Four methods have historically been successful in LST area management: acaricidal treatment of area vegetation, habitat modification, eradication or exclosure of hosts, and various types of self-medication of the hosts (Sonenshine 1993b). Acaricides that have proven successful for management of LSTs include applications of malathion, dioxathion, and toxaphene with lindane (Barnard et al. 1983) and pyrethroids in the form of sprays, dips and impregnated ear tags (Taylor et al. 1984). Successful integrated pest management strategies have included prescribed burning of pine forests (Hoch et al. 1972, Davidson et al. 1994b) and pasture rotation (Barnard et al. 1983). Because the

white-tailed deer is the most important wild host of LSTs (Bishopp & Trembley 1945, Bloemer et al. 1988, Apperson et al. 1990), the success of excluding or eradicating deer from the area of concern also has been investigated. Deer exlcusion methods have included repellents and fencing which were moderately successful (Bloemer et al. 1986, 1990), but can be a laborious, expensive, time consuming, and often unappealing option. The most desirable of these four methods of LST management described by Sonenshine (1993b) is self-medication of the host since it requires the least amount of time and manpower. Research involving this type of management began extensively in the late 1980s with the parasitic endectocide ivermectin (Miller et al. 1989, Pound et al. 1996, Hutchison 1995, Marsland 1997).

A new class of macrocyclic lactones known as avermectins was discovered while screening for anthelmintic agents (Egerton et al. 1981). The compounds were fermentation products of *Streptomyces avermitilis* MA – 4680 (NRRL 8165), an actinomycetic soil organism (Burg et al. 1979, Putter et al. 1981). These compounds also exhibited activity as a systemic insecticide-acaricide on several pests of veterinary importance (Putter et al. 1981, Drummond & Miller 1984, Drummond 1985). The commercially available version of this class of compounds was ivermectin (22, 23-dihydroavermectin B1) (Halley et al. 1993). In reviews of published data, Drummond & Miller (1984) and Drummond (1985) noted that ivermectin effectively managed the mites, ticks, lice, and parasitic and manure-breeding flies of cattle, horses, sheep and swine.

Several methods for administering ivermectin to LST hosts have resulted in excellent management. Both oral delivery and subcutaneous injections of ivermectin were found to be highly effective on LSTs and other tick species; at times 100% control was achieved (Drummond et al. 1981,Wilkins et al. 1981, Lancaster et al. 1982a, 1982b, Miller 1984). Wilson et al. (1991) report that those LSTs that fed on ivermectin-treated cattle ingested lower amounts of blood than those on untreated cattle. Drummond et al. (1981) describes successful prevention of engorgement and reproduction of six tick species including LSTs using a sustained-release bolus. The bolus is designed to remain in the reticulum for an extended amount of time, releasing a fixed daily dose of the drug. This technique is highly effective against LSTs and less time consuming than an alternative of daily subcutaneous injections. Most recently, Miller et al. (1998) developed a bioabsorbable, injectable microsphere formulation of ivermectin that releases a fixed dose over an extended amount of time, much like the sustained-release bolus. This technique resulted in 100% control of LST larvae on treated cattle for 8 weeks after injection and 75, 57, 46 and 44% over the following 4 weeks (Miller et al. 1998).

Injections, oral delivery and sustained-release boluses worked well for confined animals that could be corralled for treatment. However, treatment of wild animals, particularly white-tailed deer, was virtually impossible with these methods. Development of self-medicating devices that could administer an appropriate amount of ivermectin for an extended time to deer without human interference was necessary. Two primary strategies have been employed to successfully treat wild white-tailed deer with

ivermectin: distribution of medicated edible baits or self-treatment devices that topically treat animals feeding from them (Pound et al. 1996).

In 1989, corn treated with ivermectin was fed to confined white-tailed deer at a concentration of 35-50 μg/kg body weight/day (Miller et al. 1989). The results of this experiment were 100% and 90% control of LST adults and nymphs, respectively (Miller et al. 1989). Based on the success of LST control with orally administered ivermectin, Pound et al. (1996) fed confined deer whole kernel corn treated with 10 μg/0.45 kg corn/day by means of a solar-powered, electronically timed, automatic deer feeder. This method supplied 83, 92.4 and 100% control of LST adults, nymphs and larvae, respectively. Success with this program demonstrated the feasibility of placing automatic deer feeders filled with ivermectin-treated corn in any area where deer presence leads to a LST pest population.

In 1994, a device was patented that employed the optional strategy of topical self-treatment of white-tailed deer with Amitraz (Pound et al. 1994). This method apparently provides a similar amount of tick management as distributing ivermectin-baited corn; however, current literature reveals no detailed studies specifically for LSTs. Sonenshine et al. (1996) describes successful management of LSTs as well as *I. scapularis* with a similar device; however, the acaricide in this case was 'Liquiduster', an oily substance containing 1% concentration of the pyrethroid permethrin. Hunter-killed deer had three times more *I. scapularis* on them in the non-treatment area than in the treatment area. Management of LSTs on penned goats reached 86.4% after 4 days exposure to the self-treatment device.

iv. Lone Star Tick Population Monitoring Tools

Two of the most widely used methods for sampling LST populations, flagging and CO_2 bait trapping, take advantage of the methods the tick uses to acquire a host. The flag apparatus stimulates LSTs that are questing on vegetation to cling to the fabric when dragged over them (Sonenshine et al. 1966, Kinzer et al. 1990). The CO_2 bait trap, or dry ice trap, takes advantage of the LST's chemo-attraction to fluctuations in the level of CO_2 in its environment (Wilson et al. 1972). Both methods can serve as excellent (if somewhat different) estimators of relative LST populations in different areas.

Flagging, or dragging, typically employs a square piece of fabric that is attached to a rod along one side (Sonenshine et al. 1966, Clymer et al. 1970). The rod is attached on both sides by a long nylon cord which serves as a handle for the sampler. When the device is dragged behind the sampler, questing LSTs cling to the fabric. Estimates of LST populations are made by dragging the cloth a specified length and identifying any ticks found to stage or sex. Data are easily acquired with this method, which has been popular for decades (Milne 1943, Sonenshine et al 1966, Sonenshine & Levy 1971, Clymer et al. 1970, Kinzer et al. 1990, Schmidtmann et al. 1994). However, heavy understory and thick layers of leaf litter can make dragging inefficient in woodland habitats, thus creating a great deal of variability in collection data (Hair & Howell 1970, Wilson et al. 1972). As a consequence of this variability, an alternative method of sampling LSTs was developed by using CO_2 administered as dry ice.

Chemo-attraction to CO_2 was first demonstrated in the tick genera *Dermacentor*, *Ixodes*, and *Ornithodoros* (Garcia 1962). The research was inspired by previous observations of mosquito and flea CO_2 attraction. Several economically important tick species, including *A. americanum*, *D. andersoni* Stiles, *D. albipictus* (Packard), *I. scapularis*, and *I. ricinus* (L.), have since been shown to be attracted to increased atmospheric levels of the gas (Garcia 1965,1969, Wilson et al. 1972, Gray 1985, Ginsberg & Ewing 1989b).

Trapping ticks with sublimation of dry ice was first employed by Garcia (1965). The dry ice trap as a means of capturing *A. americanum* was first studied to compare its effectiveness to the established method of flagging (Wilson et al. 1972). The study demonstrated a 50:1 trap/flag ratio for adults and a 6:1 nymph ratio. The effective range of the trap was determined to be 25 square meters when a 1 kg block of dry ice was allowed to sublimate for 24 hours. Later studies revealed that less dry ice was sufficient for equivalent results (Koch & McNew 1982). This method became popular because it reduced the variability that occurred with flagging. Because the gas was distributed throughout the entire range of the trap, LSTs were captured that physically could not be captured with flags. The results of this and other similar studies indicate that dry ice trapping reduces variability in LST collection data and the time and manpower required (Wilson et al. 1972, Ginsberg & Ewing 1989a, Kinzer et al. 1990). Dry ice trapping also reveals LST activity earlier in the season than flagging; LSTs have not ascended vegetation early in the season which makes flagging useless, but they are responsive to CO_2 (Kinzer et al. 1990).

Climatic conditions must be favorable to acquire reliable data when using the dry ice trap to sample LST populations. Rain inhibits dispersion of the gas and LST activity. No LST response was noted by Wilson et al. (1972) at temperatures below 10^0 C in eastern Oklahoma. As long as conditions are appropriate, LSTs are easy to capture with dry ice traps. Ginsberg & Ewing (1989a) found the LST to be much faster and more aggressive in response to dry ice stimulation than *I. scapularis*, another tick commonly sampled with dry ice traps. The speed and aggression in response to CO_2 that LST demonstrates allows investigators to leave traps out for as little as one hour with favorable results (Hutchison 1995, Marsland 1997). Modern traps are based on the design of Garcia (1965) or Wilson et al. (1972), but vary greatly based on the requirements of the particular experiment. They all employ the use of dry ice which sublimates from the center of a trap (Ginsberg & Ewing 1989a).

v. Research Objectives

In 1993 an outbreak of HME occurred in Fairfield Glade (FFG), TN, on the Cumberland Plateau (Standaert et al. 1995). From 1994 to 1996, a concentration of 250 mg ivermectin / 22.7 kg whole kernel corn was administered to white-tailed deer to management the LST in FFG (Hutchison 1995, Marsland 1997). The current study continued this research using 500 mg ivermectin / 22.7 kg whole kernel corn from 1997 to 1999 in a new treatment area in FFG. The objectives of this study were 4-fold: 1) to determine the level of management of the higher ivermectin concentration, 2) to compare

the efficiency of the two concentrations relative to one another, 3) to monitor the rate of return of the lone star tick population in the area of the 1994 to 1996 treatment, and 4) to determine the efficiency of two sampling methods, carbon dioxide traps and cloth flags, relative to one another for sampling LST adults and nymphs.

During the 1993 HME outbreak, golfers that went into woods to retrieve balls were more likely to contract HME than those golfers that dropped a new ball (Standaert et al. 1995). The objective of this study was to determine the distribution of all free-living LST stages in immediately adjacent habitats. Sites were on golf courses in the original focus of the HME outbreak and nearby roadside areas adjacent to woodlands.

CHAPTER II

LONE STAR TICK MANAGEMENT WITH IVERMECTIN AND COMPARATIVE EFFICACY OF CO_2 TRAPS AND CLOTH FLAGS AS SAMPLING TOOLS

i. Introduction

The lone star tick (LST), or *Amblyomma americanum* (L.), is well distributed throughout most of the southeastern United States and Oklahoma and Texas. Historically, the tick has been implicated as a vector of causative agents for several diseases of medical and veterinary importance in the United States including tularemia, RMSF, American Q Fever, Bullis fever, tick paralysis and Potomac horse fever (Bishopp & Trembley 1945, Hair & Howell 1970). Recent investigations have implicated the LST as a vector of emerging diseases in the United States including *Ehrlichia chaffeensis* Anderson, Dawson, Jones & Wilson, the tentatively named *Borrelia lonestari*, and an as yet unnamed *Ehrlichia* species (Anderson et al. 1991, 1992, Barbour et al. 1996, Brandsma et al. 1999).

The Centers for Disease Control in Atlanta, GA, reported that an outbreak of the disease, human monocytic ehrlichiosis (HME), occurred in FFG in 1993 (Standaert et al. 1995). HME is caused exclusively by *E. chaffeensis* (Dawson et al. 1991). The disease focus was a recently opened golf course adjacent to a wildlife management area. The management area had a well established white-tailed deer population and a correspondingly high population of ticks. LSTs were particularly abundant since their

primary host is the white-tailed deer (Bishopp & Trembley 1945). LSTs have been implicated as vectors of *E. chaffeensis* (Anderson et al. 1993, Lockhart et al. 1995) and white-tailed deer are susceptible to infection by the pathogen and may be reservoirs (Dawson et al. 1994a, 1994b). In addition, experimental transmission of *E. chaffeensis* by LSTs to white-tailed deer has been demonstrated (Ewing et al. 1995). For these reasons, the LST was considered the likely vector of *E. chaffeensis* in FFG.

A reputation of infestation with disease-transmitting LSTs would have been devastating to golf revenues, so the community wanted a program that would effectively reduce risk of contracting HME. Disruption of disease cycle was necessary, but three traditional methods were considered undesirable: 1) treating the soil in the entire region with acaricides, 2) exclusion of deer from the community, and 3) reduction or eradication of deer population in the area (Marsland 1997). Therefore, a decision was made to treat the LST population by means of systemic treatment of deer with the parasitic endectocide, ivermectin.

Previous workers have described the methods used to administer ivermectin to white-tailed deer in Kerrville, TX (Pound et al. 1996), and the success in using an initial concentration of 250 mg ivermectin / 22.7 kg whole kernel corn in FFG. (Hutchison 1995, Marsland 1997). The objectives of the current study were to: 1) determine the level of management achieved using 500 mg ivermectin / 22.7 kg corn, 2) compare this level of management to the original 250 mg concentration, 3) monitor the LST population's rate of return in the area where the 250 mg concentration was administered

once treatment pressure is lifted. A fourth objective was to compare relative seasonal efficacy of two LST sampling methods: CO_2 trapping and cloth flagging.

ii. Materials and Methods

Fairfield Glade is a retirement and golf-oriented community located on the Cumberland Plateau in Cumberland Co. in eastern Tennessee. The community was developed in 1970 in a second growth mixed mesophilic forest (Marsland 1997). White pine (*Pinus strobus* L.), short leaf pine (*P. echinata* Miller), white oak (*Quercus albus* L.), hickory (*Carya* spp.), black gum (*Nyssa sylvatic*a Marsh), and sourwood (*Oxydendrum arboreum* L.) dominate the overstory. Blueberry (*Vaccimum* spp.), holly (*Ilex* sp.), wandering jew grass (*Microstegium vimineum* (Trin.) A. Camus) and sassafras (*Sassafras albidum* Nees) are primary components of a variable understory along with seedlings of the trees mentioned above and red maple (*Acer rubrum* L.). Most of the grassland in the community consists of fescue (*Festuca* spp.) and the community mows the roadsides biweekly (Mark Gnable, pers. comm.).

Two concentrations of ivermectin (250 and 500 mg / 22.7 kg whole kernel corn) were administered in two separate treatment areas during two three-year phases of the investigation. Lone star tick populations were sampled by two methods in the first treatment area (FTA) along with a nearby non-treatment area (NonTA) from 1994 to 1996. The process was repeated from 1997 to 1999 with a new treatment area (NTA) and the same NonTA. In addition the FTA was sampled from 1997 to 1999 to monitor any

rate of return of LST populations to that area. Treatment and non-treatment areas were separated from one another by a large artificial lake (Fig. 2.1).

Treatment consisted of mixing whole-kernel corn with ivermectin, a commonly used agricultural parasitic endectocide. Ivermectin was applied in the form of Ivomec® pour-on, which consists of 5 mg / ml ivermectin dissolved in 70% ethanol (Hutchison 1995, Marsland 1997). Two concentrations of ivermectin were administered between 1994 and 1999: 250 mg / 22.7 kg corn from 1994 to 1996 and 500 mg / 22.7 kg corn from 1997 to 1999. The treated corn was fed to white-tailed deer in the community by means of four, solar-powered, automatic deer feeders (Specialty Systems, Inc., Austin, TX) (Fig. 2.1). The feeders distributed corn at dawn and dusk of each day and were adjusted to have complete consumption of corn by deer with no leftover. Treatment began each year between late March and early April. Treatment ended each year in compliance with the requirement of the Food and Drug Administration for it to end 90 days before the beginning deer-hunting season which is October 31 (60 days in 1996) (Marsland 1997). Untreated corn was administered to the deer each year approximately one month before treatment initiated to acclimate the deer in the community to the presence of the feeders and the feeding schedule. The amount of corn administered during the two treatment phases of the experiment is included in Table 2.1.

Deer were extremely common throughout the area; however, no attempt was made to approximate deer numbers due to limited time. Deer presence was monitored informally near the feeders by observing scat, tracks, and, most importantly evidence of feeding. Each biweekly sampling period, the amount of corn remaining on the ground

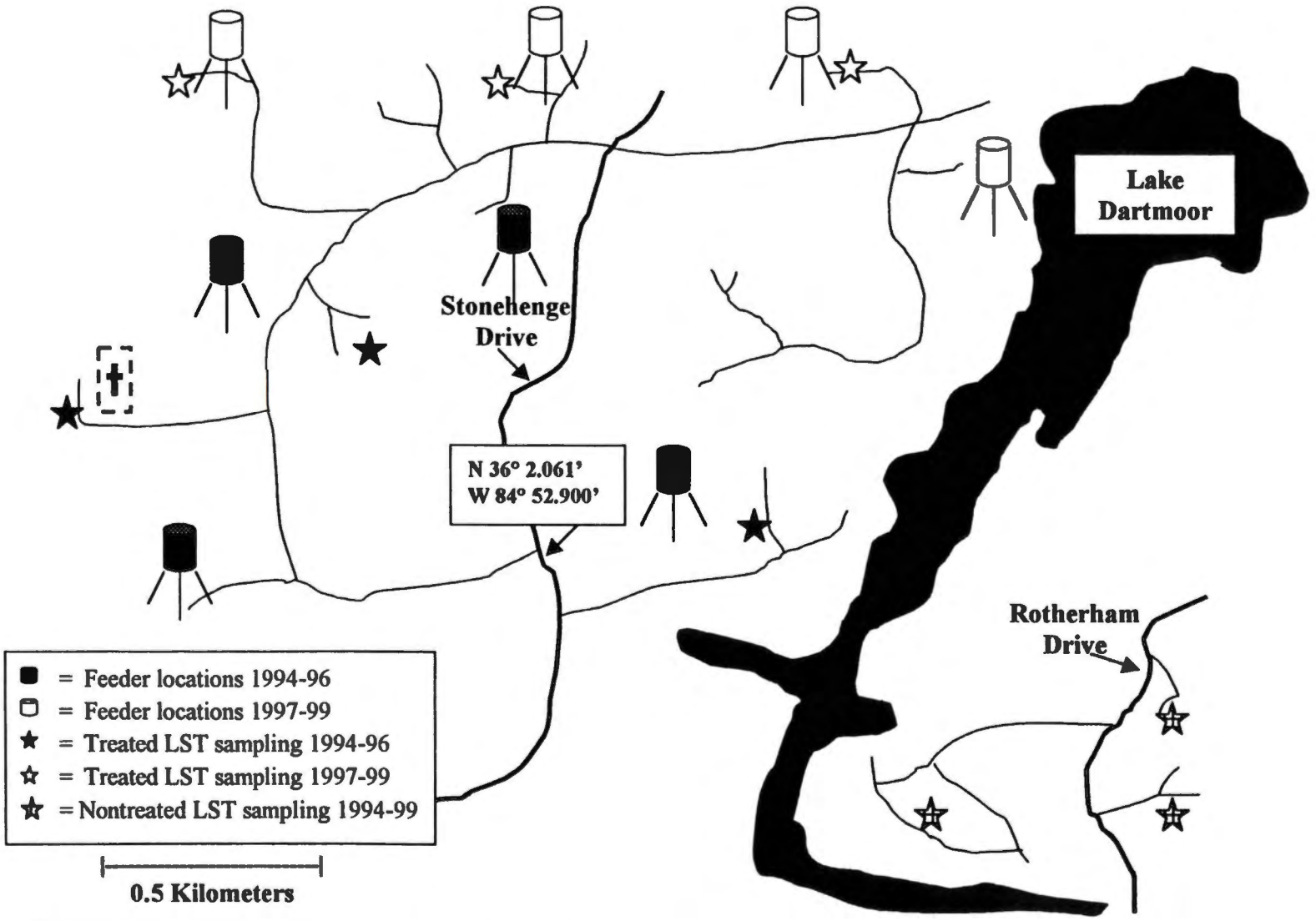

Figure 2.1 Map of the FTA, NTA and NonTA in FFG from 1994 to 1999.

Table 2.1. Start and finish dates and daily and yearly mass of whole kernel corn distributed in two ivermectin-treated corn distribution areas in Fairfield Glade, TN, from 1994-1999. *Data from 1994-1996 are from Marsland (1997). **Start date of pretreatment in 1998 was not recorded.

	Original Treatment Area			New Treatment Area		
Pretreatment	**1994***	**1995***	**1996***	**1997**	**1998**	**1999**
Start Date	11 Mar	6 Feb	17 Feb	5 Feb	?** Mar	28 Feb
Finish Date	8 Apr	13 Mar	22 Mar	15 Mar	24 Mar	7 Apr
Corn (kg)	544.8	544.8	725.7	544.8	544.8	544.8
kg/day	19.5	15.6	21.3	14.0	?**	14.0
Treatment	**1994***	**1995***	**1996***	**1997**	**1998**	**1999**
Start Date	8 Apr	13 Mar	22 Mar	15 Mar	24 Mar	7 Apr
Finish Date	31 Jul	31 Jul	31 Aug	31 Jul	31 Jul	31 Jul
Corn (kg)	1611.7	2179.2	2410	2406.2	1793.3	1952.2
kg/day	14.1	15.6	14.9	17.4	13.9	17.3

was examined and if large amounts were observed the corn dispersion rate was reduced. This adjustment was primarily done as a means of conserving treated corn since sunlight destroys the active properties of ivermectin (Halley et al. 1993). Salt blocks (22.7 kg) were placed near the feeders each year to further attract the deer to the area. In addition, black oil sunflower seeds were placed in galvanized steel garbage cans on top of the deer feeders to deter squirrels that were extracting and consuming the treated corn.

One method for sampling LSTs consisted of taking advantage of the ticks' attraction to CO_2 in the atmosphere. An approximately 0.5 kg block of dry ice was placed on a white piece of nylon fabric(1 m x 1 m) based on the design of Wilson et al. (1972). One trap was placed in a wooded area and an open, grassy, roadside area near three of the four feeders in the two treatment areas and at three sites in the NonTA during each of the two phases of the investigation (Fig. 2.1). The ice was allowed to sublimate for one hour and then removed. Any LST adults or nymphs found on the fabric were identified to sex or stage and released. Larvae are attracted to CO_2 (Wilson et al. 1972, Kinzer et al. 1990), but their extreme small size makes short term dry ice sampling for larvae ineffective. Ticks that ventured too close to the ice and perished were placed in 70% isopropyl alcohol and stored in the laboratory. Dry ice trap sampling began in March or April of each year and continued until the ticks ceased to crawl onto the traps (generally the end of July).

The second method consisted of dragging a piece of white cloth fabric behind a sampler and periodically checking the device for any attached ticks. The cloth drag piece of cloth fabric (1 m x 1 m) with one end attached to a dowel rod (approximately 1.1 m) as

described by Sonenshine et al. (1966). The dowel rod was attached at both ends to a long nylon cord which served as a handle for the sampler. Sampling occurred in the woods and open, grassy roadside areas on the same sites that the dry ice traps were placed and on the same day. The sampler dragged the device for 100 m providing a total sampling area of 100 m^2 in each wooded and grassy area at each site (Fig. 2.1). Drags were checked every 10 m for any LSTs present. Captured LSTs were identified to sex or stage and released in the same 10 m^2 area they were found. Cloth drag sampling began in March or April of each year and continued until the last larval LSTs were found (generally the end of October of each year). Because CO_2 trapping for one hour is an ineffective method for sampling LST larval masses, a second 100 m drag counting only larval masses was performed in each area when larvae were first observed each year.

Statistical analysis was performed in SAS using a variety of procedures. Raw data often were not normally distributed, due primarily to several instances when no LSTs were captured. A natural log or a double natural log transformation was necessary in these cases to produce acceptable normality. Nymphal LST data recordings larger than 200 were omitted from analysis, because they were determined to be outliers. Normality indices were examined with PROC UNIVARIATE (SAS 1989). For all LST sexes and stages analysis of variance (ANOVA) and least square mean separation were performed with PROC MIXED (SAS 1997). Two models were used in the analysis. For determining the impact of treatment on the LST population, $y_{ijkl} = a_i + b_j + c_k + ab_{ij} + ac_{ik} + bc_{jk} + abc_{ijk} + d_l(a_i) + e_{ijkl}$ was used, where y = LST sex or stage, a = year (1994 to 1999), b = sampling method (CO_2 trapping or flagging) , c = cover (woods or grass), d =

treatment (non-, previous or new), and e = residual error. For determining efficacy of sampling methods, $y_{ijk} = a_i + b_j + c_k + ab_{ij} + abc_{ijk} + e_{ijk}$, y = LST sex or stage, where a = month (March-August), b = sampling method (CO_2 trapping or flagging) , c = cover (woods or grass), and e = residual error. For both ANOVA and LSM, decisions were made using $\alpha = 0.05$.

iii. Results

Females

The yearly changes in the numbers of female LSTs, and the results of the analysis are found in Figure 2.2. From 1994 to 1996, a significant difference in mean number of females was found between the FTA and NonTA ($p = 0.006$). Means within the FTA did not differ over the three-year period, but there was a general downward trend. Means within the NonTA also did not differ during the same time period and no downward trend was observed. During 1994 the FTA mean was larger than the NonTA mean, but by 1995 and 1996, there was no difference in the FTA and NonTA means. The comparison of FTA means from 1997 to 1999 with the FTA means from 1994 to 1996 showed a significant decrease in the number of females collected ($p = 0.0466$). However, by 1999 the mean number of female LSTs increased to a level that was equivalent to the 1996 mean.

From 1997 to 1999, a significant difference was found between the NTA and NonTA ($p = 0.0251$). Means within the NTA did not differ over the three-year period,

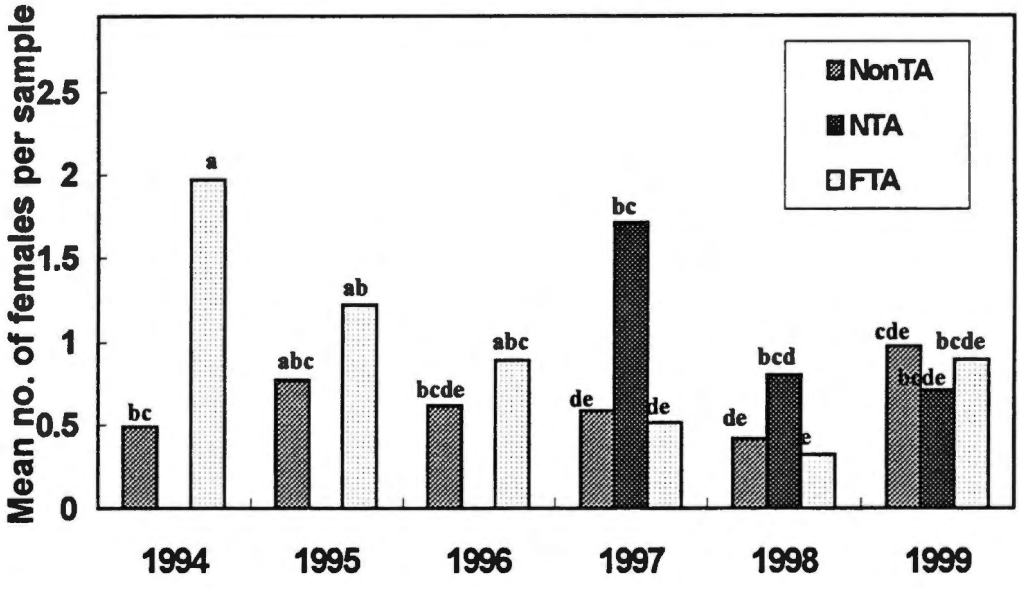

Figure 2.2. Mean number of LST females per sample in FTA, NTA and NonTA in FFG from 1994 to 1999. Reported means are non-transformed while letters indicate differences in means after a double natural log transformation ($p < 0.05$).

but there was a general downward trend. Means also did not differ within the NonTA, but no downward trend was observed. At the beginning of treatment in the NTA in 1997, the mean was larger than the NonTA mean, but by 1998 and 1999, the NTA and NonTA means were equivalent. The estimate of means comparing the level of management in the NTA from 1997 to 1999 to the level of management in the FTA from 1994 to 1996 was showed no differences ($p = 0.7807$).

Males

The yearly changes in the numbers of male LSTs, and the results of the analysis are seen in Figure 2.3. From 1994 to 1996, a significant difference was found between the FTA and NonTA ($p = 0.001$). Within the FTA the 1995 mean was equivalent to both the 1994 and 1996 means, but the 1994 mean was larger than the 1996 mean and there was a general downward trend. Within the NonTA the 1994 mean was equivalent to both the 1995 and 1996 means, but the 1995 mean was larger than the 1996 mean. During 1994 the FTA mean was larger than the NonTA mean, but by 1995 and 1996, there was no difference in the FTA and NonTA means. The comparison of FTA data from 1997 to 1999 with the FTA data from 1994 to 1996 showed a significant reduction in the number of males collected ($p = 0.0036$). The 1997, 1998 and 1999 were equivalent to the 1996 mean.

From 1997 to 1999 differences between the NTA and NonTA approached significance ($p = 0.0658$). Within the NTA all means were equivalent. In 1997 and 1998,

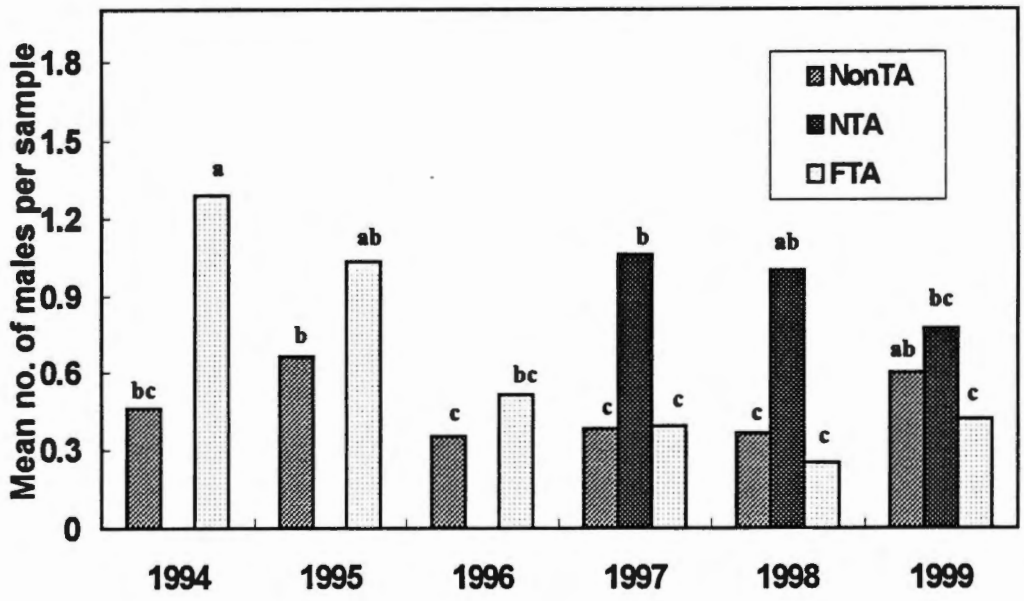

Figure 2.3. Mean number of LST males per sample in FTA, NTA and NonTA in FFG from 1994 to 1999. Reported means are non-transformed while letters indicate differences in means after a double natural log transformation ($p < 0.05$).

the NTA mean was larger than the NonTA mean. Within the NonTA the 1999 mean was larger than the 1997 and 1998 means. In 1999 the NTA and NonTA means were equivalent. The estimate of yearly means comparing the level of management in the NTA from 1997 to 1999 to the level of management in the FTA from 1994 to 1996 was showed no differences ($p = 0.7725$).

Nymphs

The yearly changes in the numbers of nymphal LSTs, and the results of the analysis are seen in Figure 2.4. From 1994 to 1996, a no difference was found between the FTA and NonTA ($p = 0.8830$). Within the FTA the 1994 and 1995 means were larger than the 1996 mean and there was a general downward trend. Within the NonTA the 1994 mean was larger than the 1995 and 1996 means, and there was a general downward trend that was not as steep as in the FTA means. During 1994 the FTA and NonTA means were equivalent. In 1995 the FTA was larger than the NonTA. In 1996 the NTA was smaller than the NonTA. The comparison of FTA data from 1997 to 1999 with the FTA data from 1994 to 1996 showed no difference ($p = 0.9839$). The 1997 and 1998 means were equivalent to the 1996 mean and by 1999, the mean number of nymphs was larger than the 1996 mean.

From 1997 to 1999, a difference was found between the NTA and NonTA ($p = 0.0049$). Within the NTA the 1998 mean was smaller than the 1997 and 1999 means. Within the NonTA, the 1997 and 1998 means were smaller than the 1999 mean and there was an general upward trend. In 1997 the NTA mean was larger than the NonTA mean.

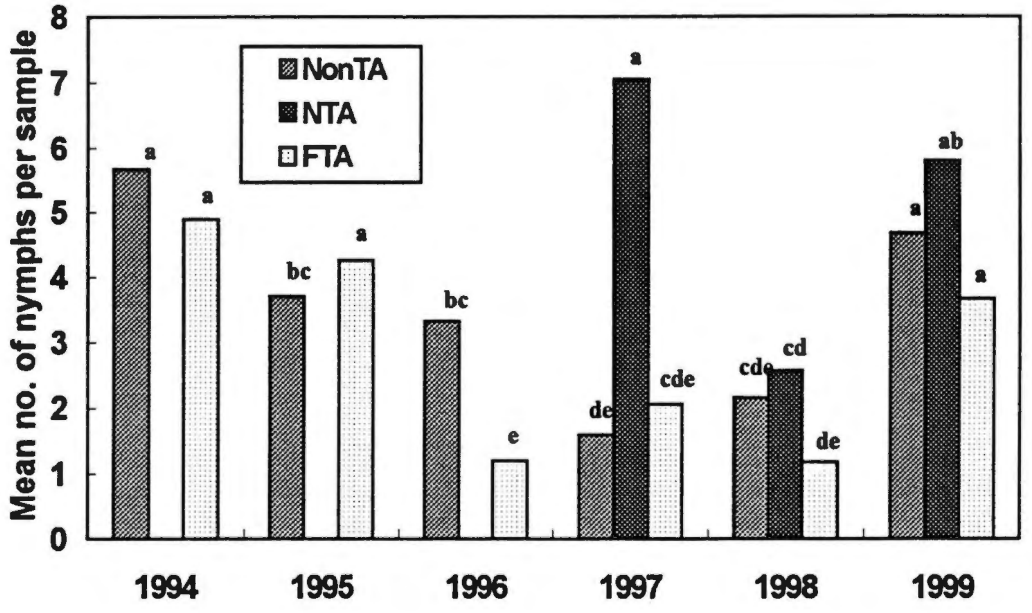

Figure 2.4. Mean number of LST nymphs per sample in FTA, NTA and NonTA in FFG from 1994 to 1999. Reported means are non-transformed while letters indicate differences in means after a natural log transformation ($p < 0.05$).

In 1998 and 1999, the NTA and NonTA means were equivalent. The estimate comparing the level of management in the NTA from 1997 to 1999 to the level of management in the FTA from 1994 to 1996 was significant ($p = 0.0304$).

Larvae

The yearly changes in the numbers of LST larval masses (1999 data are not included), and the results of the analysis are seen in Figure 2.5. From 1994 to 1996, a significant difference was found between the FTA and NonTA ($p = 0.0035$). Within the FTA all means were equivalent, but there was a general downward trend. Within the NonTA the 1994 mean was smaller than the 1995 and 1996 means. During 1994 the FTA and NonTA means were equivalent, even though there were approximately four times more females in the FTA than NonTA (Fig 2.2). In 1995 and 1996 the FTA means were smaller than the NonTA means. The comparison of FTA data from 1997 to 1998 with the FTA data from 1994 to 1996 showed no difference ($p = 0.8686$). The 1997 and 1998 means were equivalent to the 1996 mean.

From 1997 to 1998, the NTA and NonTA did not differ ($p = 0.4335$). Within the NTA the 1997 and 1998 means were equivalent. Within the NonTA, the 1997 mean was smaller than the 1998 mean. In 1997 the NTA mean was equivalent to the NonTA mean. In 1998 the NTA mean was smaller than the NonTA mean. The estimate comparing the level of management in the NTA from 1997 to 1999 to the level of management in the FTA from 1994 to 1996 was close to significant ($p = 0.0675$).

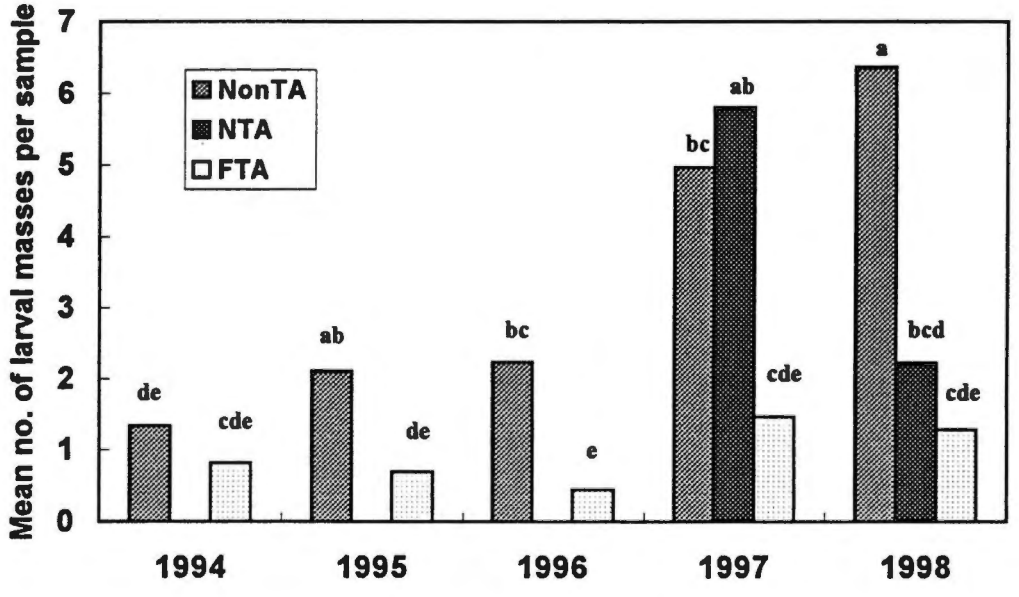

Figure 2.5. Mean number of LST larval masses per sample in FTA, NTA and NonTA in FFG from 1994 to 1998. Reported means are non-transformed while letters indicate differences in means after a natural log transformation ($p < 0.05$).

Efficacy of CO_2 Traps and Cloth Flags

Seasonal efficacy of CO_2 traps and cloth flags as LST sampling tools was determined. The data used were from the NonTA from 1994 to 1999. Larval LSTs could not be analyzed, because they were only sampled with cloth flags. Three-way interaction of month, sampling technique and cover was significant for female, male and nymphal LSTs ($p = 0.0006$, 0.0057, and 0.0001, respectively). Females were captured most frequently from March to June with CO_2 traps in the wooded areas (Fig. 2.6). In March, the mean number of females captured in grass by CO_2 traps was equivalent to the same method in wooded areas (Fig. 2.6). No significant differences in females were observed for sampling method or cover after June. Males were captured most frequently in March and April in wooded areas with CO_2 traps (Fig. 2.7). No significant differences in males were observed for sampling method or cover after June (Fig. 2.7). Nymphal LSTs were captured most frequently from May to August in the wooded areas by means of cloth flags (Fig. 2.8). In April, the most nymphs were captured in wooded areas with CO_2 traps. Non-transformed means and letters representing significant differences for each LST sex and stage sorted by sampling technique and cover are presented in Table 2.1.

iv. Discussion

Analysis of the six-year ivermectin treatment program revealed that the LST population was successfully managed in FFG. However, some discrepancies in the data may be partially explained by natural fluctuations in the size of the LST population.

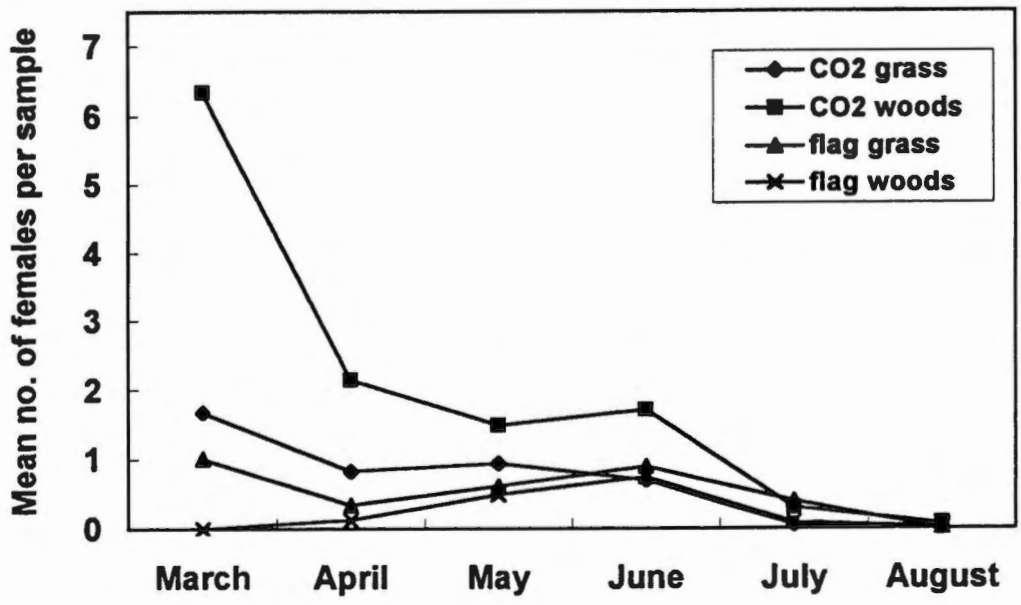

Figure 2.6. Mean number of LST females by sampling method, cover and month in the NonTA of the FFG ivermectin treatment program from 1994 to 1999. Statistical comparisons are found in Table 2.2.

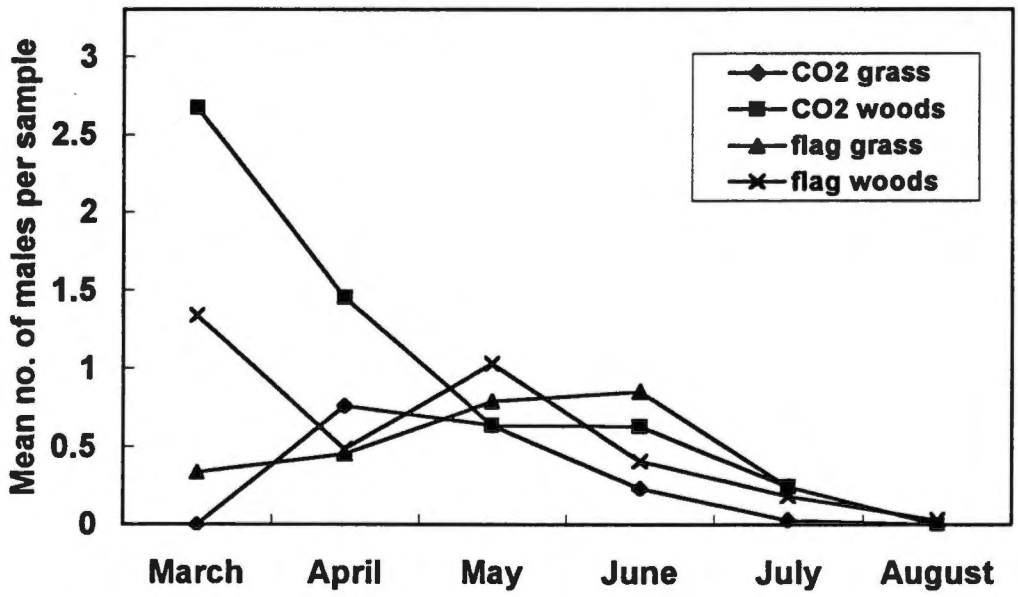

Figure 2.7. Mean number of LST males by sampling method, cover and month in the NonTA of the FFG ivermectin treatment program from 1994 to 1999. Statistical comparisons are found in Table 2.2.

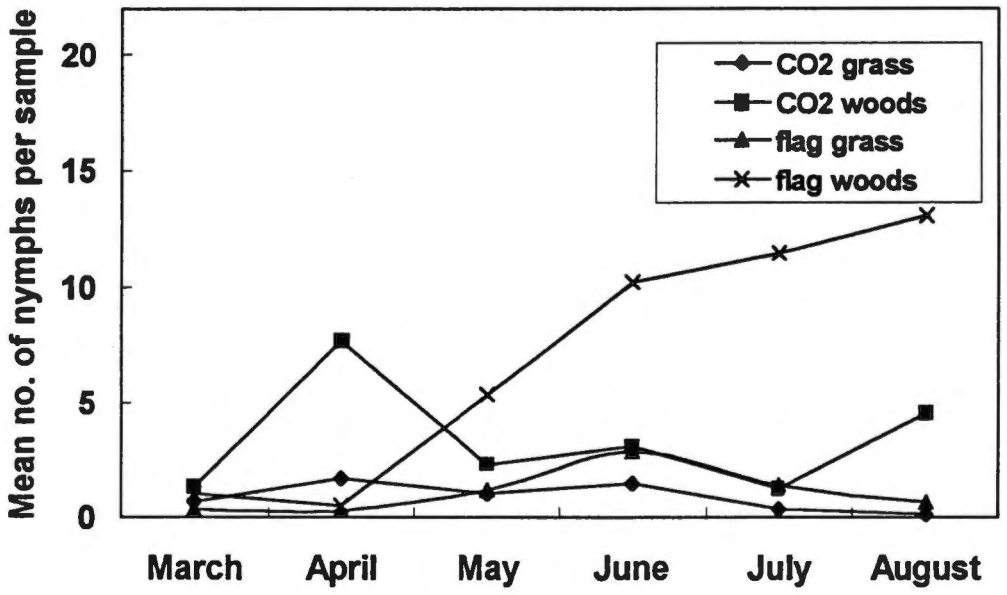

Figure 2.8. Mean number of LST nymphs by sampling method, cover and month in the NonTA of the FFG ivermectin treatment program from 1994 to 1999. Statistical comparisons are found in Table 2.2.

Table 2.2. Mean number of male, female and nymphal lone star ticks captured with CO_2 traps and cloth flags in wooded and open, grassy areas in the non-treatment section of the Fairfield Glade, TN, ivermectin treatment program from 1994-1999. Raw means are reported and letters represent significant differences of means after natural log transformation.

Females	March		April		May		June		July		August	
CO_2 grass	1.6667	A-E	0.8276	CDEF	0.9394	DEFG	0.6923	D-H	0.0606	JK	0.0000	JK
CO_2 woods	6.3333	A	2.1379	B	1.4848	BC	1.7037	B	0.3030	HIJK	0.0741	JK
flag woods	1.0000	B-K	0.3333	GHIJ	0.6061	EFGH	0.8889	CD	0.3939	HIJK	0.0000	K
flag grass	0.0000	F-K	0.1212	IJK	0.4848	FGHI	0.7407	DEF	0.0909	IJK	0.0278	JK
Males												
CO_2 grass	0.0000	E-J	0.7586	CDE	0.6364	DEGH	0.2308	GHIJ	0.0303	J	0.0000	J
CO_2 woods	2.6667	A	1.4483	B	0.6364	DEG	0.6296	CDEG	0.2424	HIJ	0.0000	J
flag woods	0.3333	B-J	0.4545	D-I	0.7879	CDF	0.8519	CDE	0.2424	GHIJ	0.0000	J
flag grass	1.3333	ABCD	0.4848	EGHI	1.0303	BC	0.4074	D-I	0.1818	IJ	0.0278	J
Nymphs												
CO_2 grass	0.6667	B-H	1.6897	EF	1.0000	EFG	1.5000	DEF	0.3636	GH	0.1481	H
CO_2 woods	1.3333	B-H	7.7241	BCD	2.2727	CDE	3.1111	BCD	1.2727	EFG	4.5556	CDE
flag woods	0.3333	C-H	0.3030	GH	1.2121	EF	2.8519	BC	1.3636	DE	0.6389	FGH
flag grass	1.0000	B-H	0.4848	GH	5.3871	B	10.2593	A	11.4545	A	13.1111	A

Analysis of larval masses is incomplete and therefore the reported results were examined with increased caution. The true impact of the 500 mg ivermectin / 22.7 kg corn treatment concentration on LST larval masses will not be evident until the end of this year. In addition, the analysis of sampling technique revealed CO_2 traps and cloth flags may both be necessary to completely sample all free-living stages of a given LST population.

The importance of examining rates of decline of a given LST sex or stage rather than just mean differences is most prominent in the female data. Figure 2.2 reveals that the actual treatment means from 1994 to 1996 and 1997 to 1999 do not differ significantly across each three-year time span. However, the estimate analyses comparing the means in both the FTA and NTA to the means in the NonTA are significant. Understanding the reason for the significant estimate analysis requires examining the FTA and NTA means relative to the NonTA mean within each year. The 1994 FTA female mean does not differ from the 1996 FTA female mean. However, the 1994 FTA female mean does significantly differ from the 1994 NonTA mean and the same two means do not differ in 1996. This demonstrates that size of the female LST population in the two treatment areas decreased to approximately the size of the NonTA population within each three-year period (1994 to 1996 for the FTA and 1997 to 1999 for the NTA). This same general relationship also is evident in the male FTA and NTA data (Fig. 2.3).

From 1997 to 1999, male and female LST populations in the FTA remained significantly at or below their mean numbers in 1996, the end of the first treatment. This

maintenance of low LST adult means in the FTA after treatment ended, complemented with the fact that NonTA adult means have stayed basically the same over the six-year period, suggests that treatment of 250 mg / 22.7 kg whole kernel corn to white-tailed deer in a woodland community may have at short-term management effect on a LST population.

The final estimate dealt with the relative LST management success of 250 and 500 mg / 22.7 kg whole kernel corn fed to the deer in FFG. Female and male LST analyses showed no differences between the level of management of the two concentrations. This lack of significance suggests that no additional management was provided by the 500 mg concentration. The 250 mg concentration probably provides as a much LST management as is possible when treating wild white-tailed deer systemically. Again, nymphal and larval LSTs were significant for this estimate and this situation will be considered with the other discrepancies of nymphal and larval LST data.

For all of the estimates, either nymphal or larval LSTs or both had the opposite significance relationship to adults. The most easily explained are the two estimates comparing level of management in the FTA and NTA relative to NonTA. Figure 2.4 shows that the 1994 nymphal LST mean in the FTA was significantly higher than the 1996 nymphal LST mean in the FTA. However, the same is true in the NonTA. This 1994 to 1996 reduction in means suggests that there was a natural decline in the LST population and therefore the rate of decline in the FTA is not readily observable. However, in 1994 the FTA and NonTA nymphal LST means were the same, but the 1996 FTA mean was much smaller than the NonTA mean. The opposite situation existed for

nymphal LSTs in the NTA which is why the rate estimate was significant. The 1997 NTA nymphal mean was much larger than the NonTA mean; the 1999 NonTA nymphal mean has naturally increased, but the NTA 1999 remained the same as the 1997 mean. The non-significant estimate of larval mass means in comparison of NTA and NonTA for 1997 and 1998 will probably be significant when 1999 data are included; larval masses differed between FTA and NonTA in 1994 to 1996 and all other stages were significant for the NTA and NonTA 1997 to 1999 comparison (Fig. 2.5).

The fact that FTA, NTA and NonTA nymphal means did not from one another and were significantly higher than the 1998 means suggest that survival conditions were favorable for LST nymphs as a whole in 1999 (Fig. 2.4). This increase was not as extreme in female and male LST 1999 data, but there was a significant increase in the NonTA in 1999 for both sexes (Fig. 2.2 and 2.3, respectively). The increase in nymphal means in the FTA and NTA in 1999 and lack of increase in adult means in the same areas, complemented with the fact that there were no differences in the 1994 to 1996 nymphal means, suggests that nymphs are not as susceptible to ivermectin as adults. Decreased susceptibility of nymphal LSTs to ivermectin cannot be determined conclusively from this study and warrants further research.

A recommendation for future research in the management of LSTs with treated corn stems from the necessity to cease treatment on July 31 of each year in accordance with Food and Drug Administration regulations. An effective compound that has no withdrawal is desirable. The compound moxidectin has been shown to be equally as effective as ivermectin in controlling internal parasites (Costa et al. 1998) and it has been

shown to manage ticks (Sibson 1994). In addition, it is available for oral delivery like ivermectin and has no withdrawal period. However, no published research addresses the efficacy of moxidectin on a wild vertebrate population like this ivermectin treatment study has done with white-tailed deer. The level of LST management provided by moxidectin-treated corn fed to deer could be an excellent next step in FFG.

The results of the analysis of sampling techniques in capturing LST adults and nymphs is useful, practical information for those interested in LST population monitoring. In the month of April between 1994 and 1999 in FFG, males, females and nymphs were all captured most frequently by means of CO_2 traps in wooded areas (Fig 2.6, 2.7 and 2.8, respectively). Adults also were captured most frequently by CO_2 traps in March, but not nymphs. This difference is probably due to an earlier emergence of LST adults compared to nymphs (Hair & Howell 1970). At any rate, early in the season CO_2 traps appear to be the best sampling tool. The fact that LSTs do not respond as well to cloth flags in the spring as they do to CO_2 traps may be due to most LSTs not ascending vegetation and actively questing until May.

The analysis of sampling data apparently indicated a difference in the level of aggression by males and females to CO_2 stimulus. An equal number of males were captured by both techniques in May and June (Table 2.1). For LST females, May and June means were similar for the two sampling techniques, but CO_2 traps always captured more (Table 2.1). This more extended response to CO_2 traps by females may reflect a greater level of aggression to CO_2 stimulus by females than males. *Amblyomma americanum* has been shown by Ginsberg & Ewing (1989a) to be strongly attracted to

CO_2. No one, however, has published a study comparing differences in the level of aggression by the two LST sexes to CO_2 and this warrants further investigation.

The sampling technique most successful in capturing nymphs was the cloth flag. The opposite relationship was true for similar studies by Kinzer et al. (1990) in Mississippi and Davidson et al. (1994a) in Georgia. The study by Kinzer et al. (1990) also reported that larvae were more attracted to CO_2 traps than cloth flags. This extreme difference suggests geographic variability in sampling efficacy and both techniques may be necessary for accurate analysis. As mentioned, more were captured by CO_2 traps in wooded areas in April, but this is the only month that flags captured fewer nymphs than CO_2 traps in wooded areas. For the rest of the season, far more nymphs are captured by cloth flags in wooded areas (Table 2.1). Only in August were more nymphs captured by CO_2 traps in woods than in grass with CO_2 traps or cloth flags. This significantly higher mean in August could reflect nymphs that emerged in spring that have become more desperate for a bloodmeal and are thus more attracted to CO_2 traps. Nymphs were unresponsive to CO_2 traps after August which is why later months were not included in this analysis.

Adults were most responsive to CO_2 traps in the spring and early summer months; nymphs were most responsive to cloth flags in summer and fall. In addition, larval masses were not effectively sampled with CO_2 traps when CO_2 sublimation was only allowed to occur for one hour. These observed differences in the responses of the free-living LST stages' responses to CO_2 traps and cloth flags suggest that both are necessary to appropriately estimate the size of a LST population.

CHAPTER III

ADJACENT HABITAT DISTRIBUTION OF THE LONE STAR TICK

i. Introduction

In 1993, an outbreak of human monocytic ehrlichiosis (HME) occurred in FFG (Standaert et al. 1995). The causative agent of this disease is *Ehrlichia chaffeensis* Anderson, Dawson, Jones & Wilson (Anderson et al. 1991, 1992, Dawson et al. 1991). The suspected vector was *Amblyomma americanum* L., or the lone star tick (LST), due to its previous implication as a natural vector (Anderson et al. 1993), and its abundance in this community. A management program using ivermectin to reduce the LST population in the area was initiated in 1994 (Hutchison 1995, Marsland 1997). Management efforts were successful during the administration of 250 mg of this parasitic endectocide yielding a 61% and 44% decline in female and larval populations, respectively (Marsland 1997). However, there is no guarantee that the size of the population will not increase now that the treatment program has ended.

Previous studies indicate that the largest LST populations exist in wooded areas compared to grassy areas, such as open fields (Hair & Howell 1970, Semtner et al. 1971a, 1971b, Semtner & Hair 1973a, 1973b, Sonenshine & Levy 1971). Hair & Howell (1970) contend that the low numbers of LSTs in open, grassy areas in Oklahoma are a function of seasonally low relative humidities and high temperatures. In Virginia where conditions are not as severe in the summer months, fewer LSTs were found in field

habitats than in adjacent woodlands (Sonenshine et al. 1966, Sonenshine & Levy 1971). The studies in Virginia and Oklahoma revealed contrasting results when comparing abundance of LST in different vegetative types of woodlands (Sonenshine & Levy 1971), but they all contend that survival is lowest where there is the least protection from the sun.

During the FFG HME outbreak in 1993, golfers that went out of bounds to retrieve a ball rather than dropping a new ball stood a higher likelihood of being exposed to *E. chaffeensis* (Standaert et al. 1995). The golf fairways in FFG are immediately adjacent to wooded areas with little or no grass rough which implies that the golfers were more likely to come into contact with LSTs in the woods than in the open, grassy fairways. Researchers in the northeastern United States have compared relative numbers of *I. scapularis* between woods, ecotone and lawn (Falco & Fish 1988, Maupin et al. 1991, Stafford & Magnarelli 1993, Duffy et al. 1994) and woods, ecotone and pasture (Schmidtmann et al. 1994). Hair & Howell (1970) found the highest concentration of LST nymphs and larvae in the ecotone habitat between dense woods and open fields in Oklahoma. No published study focuses on the relative distribution of LSTs between woods, ecotone and field in the southeastern United States where HME has become a problem.

A study was initiated in 1997 to assess the natural distribution of free-living LST larvae, nymphs and adults on the fairways and wooded roughs of golf course sites as well as the open, grassy and wooded areas of selected roadside sites in FFG. Sampling consisted of dragging cloth flags across adjacent 50 m transects in wooded, grassy and

ecotonal habitats. The results of this research will have great practical value to public health because they should reveal the most likely habitat type examined in this study for LST in this region.

ii. Materials and Methods

Fairfield Glade is a retirement and golf-oriented community located on the Cumberland Plateau in Cumberland Co. in eastern Tennessee. The community was developed in 1970 in a second growth mixed mesophilic forest (Marsland 1997). White pine (*Pinus strobus* L.), short leaf pine (*P. echinata* Miller), white oak (*Quercus albus* L.), hickory (*Carya* spp.), black gum (*Nyssa sylvatic*a Marsh), and sourwood (*Oxydendrum arboreum* L.) dominate the overstory. Blueberry (*Vaccimum* spp.), holly (*Ilex* sp.), wandering jew grass (*Microstegium vimineum* (Trin.) A. Camus) and sassafras (*Sassafras albidum* Nees) are primary components of a variable understory along with seedlings of the trees mentioned above and red maple (*Acer rubrum* L.).

The roadsides in FFG are composed primarily of fescue (*Festuca* spp.) and the community mows the entire area biweekly (Mark Gnable, pers. comm.). On the golf courses, the fairway and rough are composed of bluegrass (*Poa pratensis* L.) and ryegrass (*Lolium perenne* L.) except at the tree lines where the primary grass is fine-leaf fescue (*Festuca rubra* L.). The fairways are mowed five days each week with a reel and bed knife mower providing a close cut, while the rough is mowed weekly with a rotary blade (Mark Gnable, pers. comm.).

Several golf course sites were initially sampled in summer 1997 to locate areas with the largest LST populations. In early spring 1998, roadside sites were selected in the same manner. Three golf course sites and three roadside sites were selected based on these surveys and sampled for free-living LSTs biweekly from April to October 1998 and from April to June 1999 (Fig. 3.1).

Each site consisted of 50 m transects, arranged approximately parallel to the ecotone (small bushes and trees between the grassy and wooded areas of the site). The transect centerlines were separated from one another by 5 m. Four sites had five transects positioned 5 (5W) and 10 (10W) m into the woods, 5 (5F) and 10 (10F) m into the grass and one at the ecotone (E). The 10F was not sampled at two roadside sites because it was located on pavement or gravel. Figure 3.2 represents the general layout of the six sites in this study.

Drag cloths were used as sampling tools and consisted of square pieces of white flannel cloth (1 m x 1 m) as described by Sonenshine et al. (1966). The fabric was attached along one edge of the square to a dowel rod (approximately 1.1 m) with a 5 cm diameter. The dowel rod had a nylon string attached at both ends to serve as a handle for the sampler. The devices were dragged down both sides of the centerline of each 50 m transect, providing a total sampling area of 100 m^2 (Figure 3.2). Cloth drags were checked every 10 m for LSTs. The stage or sex of any captured LST was recorded, and the ticks were returned to the same 10 m^2 area. Larval masses were recorded rather than individual larvae, and captured larvae were removed with tape due to their small size.

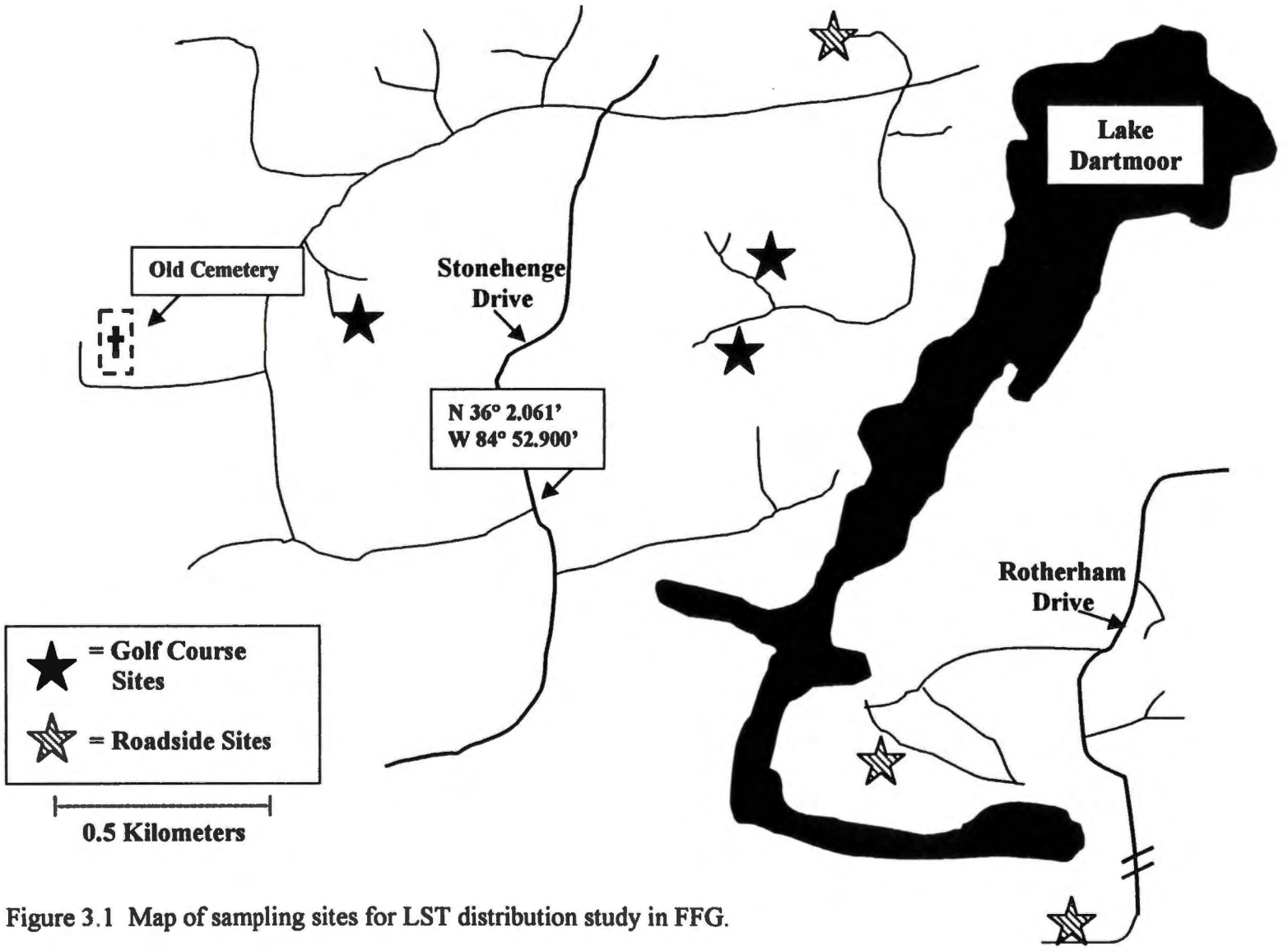

Figure 3.1 Map of sampling sites for LST distribution study in FFG.

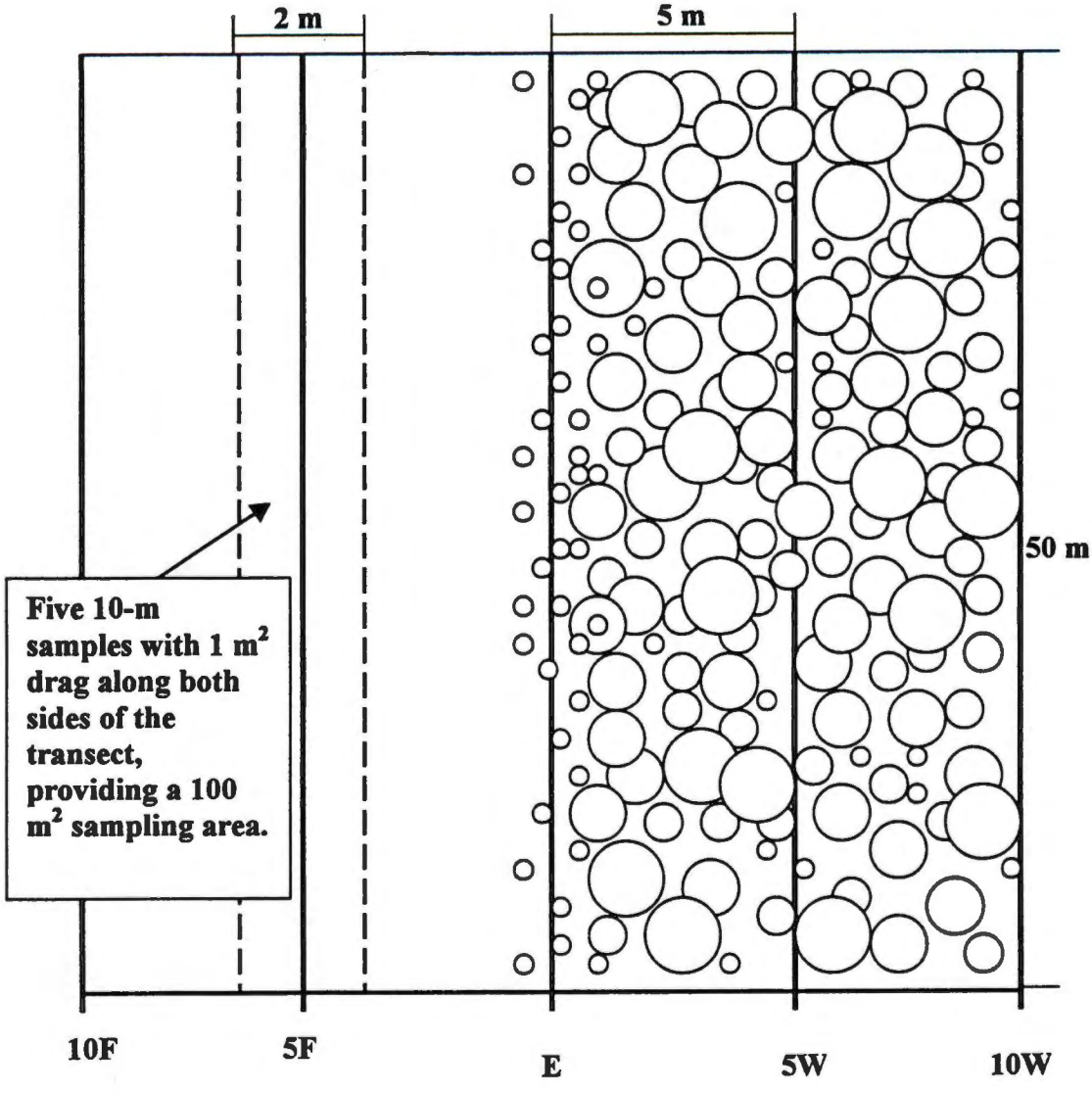

Figure 3.2. General diagram for layout of transects in FFG.

Data for nymphs and larval masses were analyzed by SAS version 6.12 (SAS Institute 1997) using a mixed models procedure (PROC MIXED) to perform a two-factor analysis of variance (ANOVA). The nymph and larval mass data were transformed using a log + 0.5 transformation to improve normality; however, means of the non-transformed data are reported. Two variables, habitat type and a categorical variable comparing data from roadside and golf course sites, were included in the analysis. Due to small numbers of captured males and females, adult data were analyzed with FET using a procedure to develop and analyze frequency tables (PROC FREQ). In this analysis, presence of an adult is assigned a value of 1 and absence of an adult is assigned a value of 0. All decisions were made using a 95% confidence interval ($p \leq 0.05$).

iii. Results

Preliminary data from 1997 included few adults so only nymphs and larval masses were analyzed, and only those sites that had more than one recorded nymph or larval mass in the entire 1997 sampling period were included in analysis. These data were eliminated to improve poor normality resulting from several sites having no detectable LST populations. Both nymphal and larval mass ANOVAs of habitat yielded significant values ($p = 0.0012$ and 0.0096, respectively). Nymphal means were largest in 10W, 5W and E (Fig. 3.3). The 5F and 10F transects did not differ from one another or E. Mean separation of larval masses data revealed the highest mean was at the 10W and the 5W, E, 5F and 10F were statistically identical to one another.

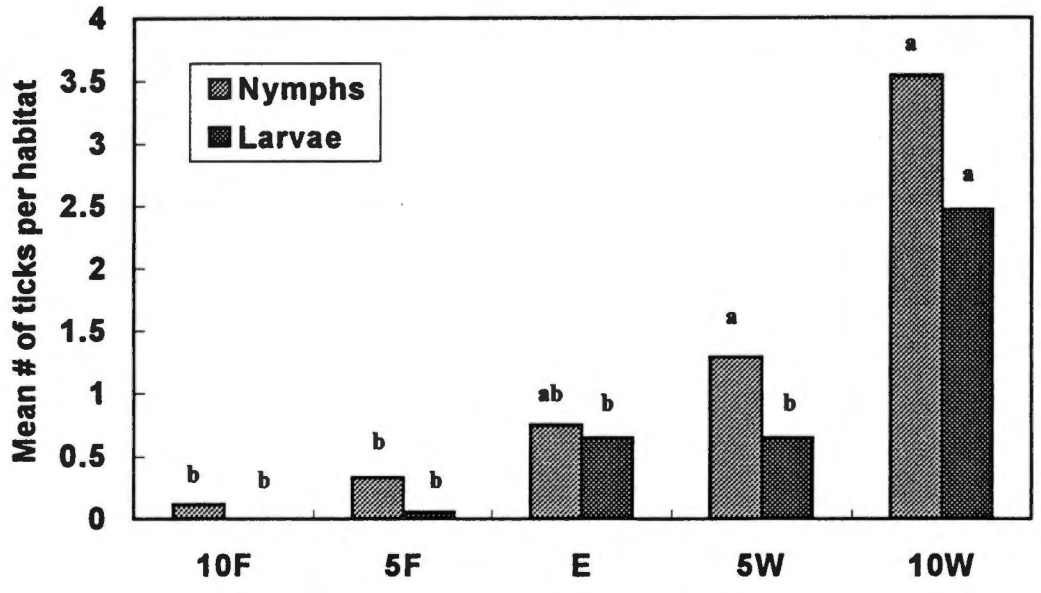

Figure 3.3. Mean number of LST nymphs and larval masses in five adjacent habitats on golf course and roadside sites in FFG during 1997. Means are non-transformed while letters represent significant differences within the nymphal or larval stages after natural log transformation ($p < 0.05$).

Larval masses differed across habitat type ($p = 0.0001$). Larval masses were most abundant in the 5W and 10W transects (Fig. 3.4). The E, 5F and 10F transects were not significantly different from one another. Golf and roadside sites did not differ ($p = 0.8956$). The interaction of site and habitat type also was not important ($p = 0.5539$). Because the golf course site data did not differ from one another, letters above each set of two bars at each habitat represent the ANOVA for habitat and not the interaction of habitat and site.

The nymphal ANOVA of habitat type had a significant p-value of 0.0001. Lone star tick nymphs were most abundant overall in the 10W transect in 1998 and 1999. The 5W transect had the second highest mean number of nymphs. The 5F transect did not differ from either E or 10F, but there were significantly more nymphs in E than 10F (Fig. 3.5). Golf and roadside sites were found to differ significantly from one another ($p=0.0140$) and the interaction of site and habitat type also was significant ($p=0.0287$). In Figure 3.5, letters are found above each individual bar and represent the ANOVA of the interaction term rather than habitat type.

In the adult analysis, only the interaction of site and habitat type could be considered with FET. At $\alpha = 0.05$, males yielded a non-significant value ($p = 0.710$) while female FET analysis produced a significant value (p 0.031). Although an ANOVA could not be performed on either males or females, the actual numbers in each type of site and habitat is plotted (Fig. 3.6 and 3.7, respectively).

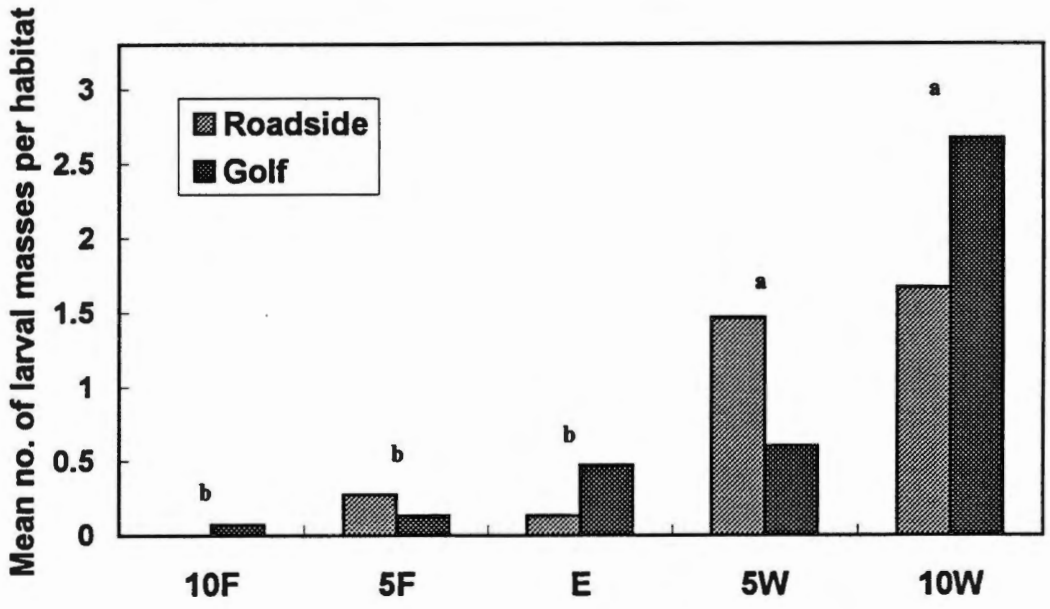

Figure 3.4. Mean number of LST larval masses in five adjacent habitats on golf course and roadside sites in FFG in 1998. Reported means are non-transformed while letters represent habitat differences, the only significant factor, after natural log transformation ($p < 0.05$).

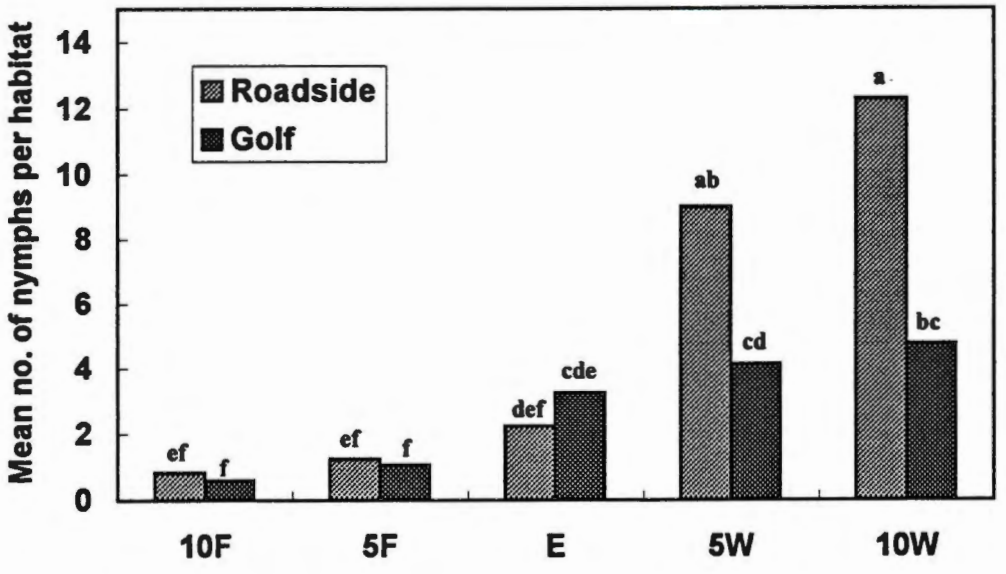

Figure 3.5. Mean number of LST nymphs in five adjacent habitats on golf course and roadside sites in FFG from 1998 to 1999. Means are non-transformed while letters represent differences after natural log transformation ($p < 0.05$).

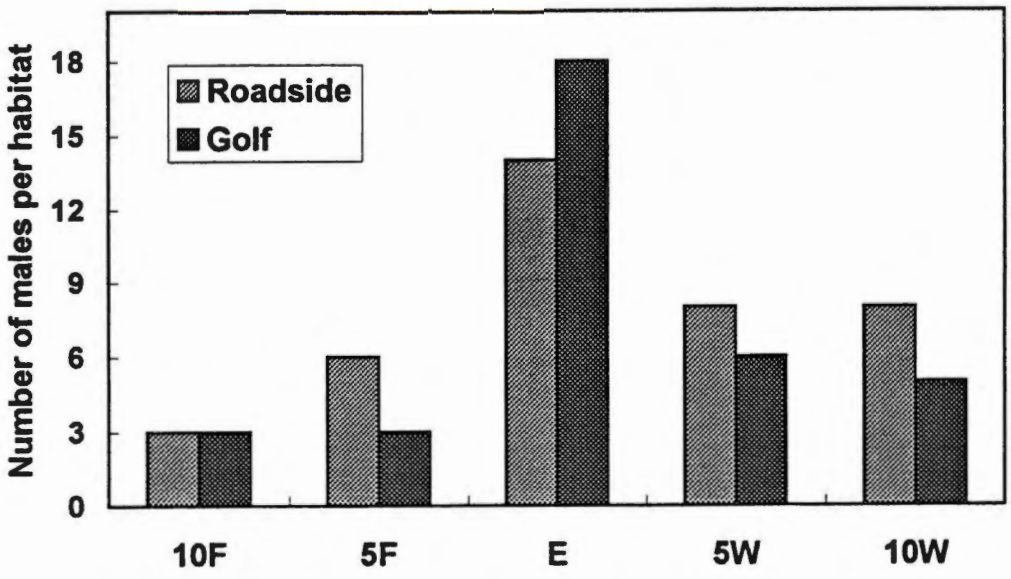

Figure 3.6. Total number of LST males in five adjacent habitats on golf course and roadside sites in FFG from 1998 to 1999.

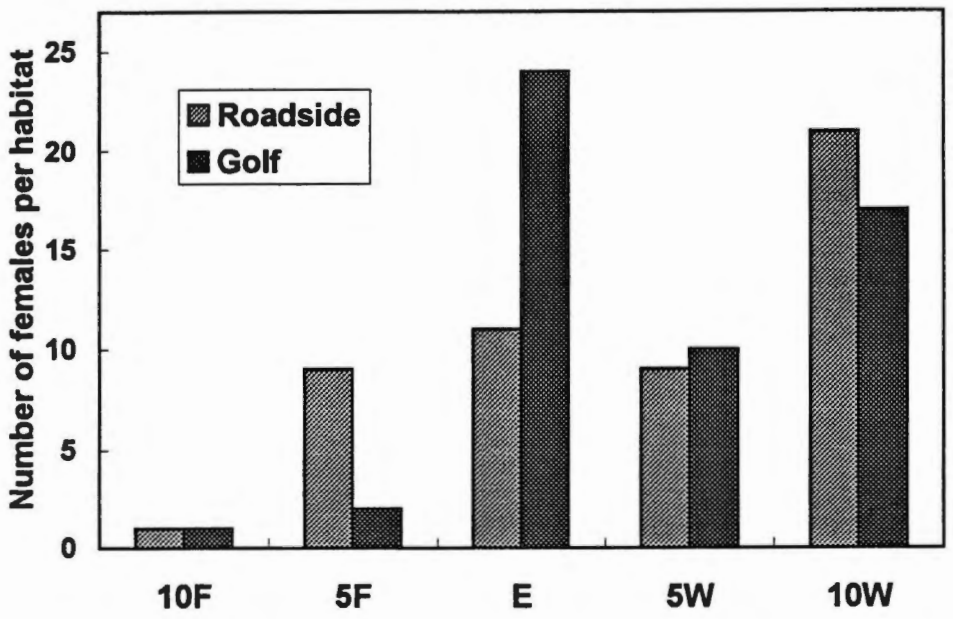

Figure 3.7. Total number of LST females in five adjacent habitats on golf course and roadside sites in FFG from 1998 to 1999.

iv. Discussion

It is intuitive that the largest LST populations are in habitats providing some degree of shade and moisture since LSTs must seek favorable habitat to avoid desiccation (Hair & Howell 1970, Semtner et al. 1971a). The general distribution of LST relative to the ecotone appears basically the same in the south-central and southeastern United States. Our results are in accord with those of Hair & Howell (1970) and Sonenshine et al. (1966) with few exceptions. LST larvae, nymphs and adults were captured at the 5F and 10F transects of almost every site, but their low numbers relative to the E, 5W, and 10W transects suggest that survival is highest in wooded areas.

The distribution of larval masses in this study is consistent with general LST biology described by Hair & Howell (1970). The engorged, detached female does not move around much and basically must lay her eggs where she lands. Also, the small size of the larvae prevents them from travelling far before they congregate in a mass on vegetation. If a female does happen to drop off in an open, grassy area that is mowed with any frequency, the larval mass will probably be disrupted by the mowing. Any larvae that survive mowing in open, grassy areas are at great risk of desiccation. Therefore, it is extremely unlikely that one will encounter many larval masses in an open, grassy area. Few larvae were found in the field habitats in this study, even in roadside areas where vegetation was often tall.

The highest concentrations of LST nymphs were found in Oklahoma by Hair & Howell (1970) in dense ecotones where vegetation height was between 1.2 and 6.1 m,

presumably as a consequence of host utilization. The height of the ecotone and woodlands in our study was similar to that of Hair & Howell (1970), but we observed the opposite nymphal distribution among E, 5W and 10W habitats. The ecotone mentioned in Hair & Howell (1970) appears to be a relatively wide area compared to the narrow man-made ecotone in our study. Areas with larger ecotones in the southeastern United States may have a similar distribution to that mentioned by Hair & Howell (1970).

Based on personal observations, the highest concentrations of LST nymphs in the E, 5W, and 10W transects were typically captured in patches of blueberry. Blueberry is not a significant part of the diet of white-tailed deer which is the primary host of LSTs (Pugh 1978), so it is curious that so many nymphs would be found there. The occurrence of nymphal concentrations in this area may be due to engorged LST larvae dropping off hosts that sleep in the area of the blueberry patches during late fall and winter months. A correlation of host sign during late fall and winter with geographic occurrence of blueberry might support this idea. This study did not quantify animal sign in the area so the idea remains a hypothesis.

The distribution pattern for adults appears different than that of nymphs and larval masses in this study. However, the small number of sampled LST adults make this observation difficult to state conclusively, because ANOVAs could not be performed on their distribution. *Ixodes scapularis* females can move much greater distances over time than nymphs of the same species (Carroll & Schmidtmann 1996). Also, LSTs were much more aggressive in response to CO_2 stimulation than *I. scapularis* (Ginsberg & Ewing 1989a). In addition, adult and nymphal *I. scapularis* on a horse pasture bordered by

woodland had a distribution similar to the one documented in this study (Schmidtmann et al. 1994). These factors suggests that differences in distribution may be a consequence of adult LSTs moving faster and more aggressively toward a CO_2 source.

The most LST males were found at E in both roadside and golf sites, but the most females were found at the golf E and golf and roadside 10W sites. Once again, the impact of the lack of 10F data for two roadside sites is probably negligible. No published information compares female and male LST response to CO_2, but females are larger, and during this study, were observed to be much more aggressive than males. The difference in female and male distributions may be a consequence of this greater aggression. Twice as many females were found in golf E than roadside E (Fig 3.5). Many people travel down the fairways in this community daily and could be attracting the females out of the woods away from other hosts, but the role that humans play in the distribution of female LSTs on golf courses is not demonstrable from this study.

The data from this study demonstrate that the habitat one is least likely to encounter LSTs of any stage in FFG is an open, grassy area. However, this likelihood is a relative risk and the ticks were frequently found on golf course roughs and fairways as well as roadside grassy areas. There were many more males and females in E for both types of sites relative to nymphs and larval masses. The large proportion of adults in E suggests that all LST stages may emerge in the woods, but depending on size and aggression toward CO_2 stimulus, they may move out of the woods into the more open, grassy areas in search of hosts. These data further stress the importance of protective clothing and repellent in recreation or any outdoor areas where ticks commonly occur.

CHAPTER IV

CONCLUSIONS

i. Ivermectin Treatment and Sampling Efficacy

Ivermectin-treated corn was an effective tool for managing the LST population in FFG. The method of treating whole kernel corn with ivermectin used in this study delivers an appropriate dose of the chemical to the bloodstream of the white-tailed deer that consume the corn in FFG. The original concentration of 250 mg ivermectin / 22.7 kg whole kernel corn was the most appropriate; the new concentration of 500 mg ivermectin / 22.7 kg whole kernel corn provided the same level of management as the 250 mg concentration. Means of all stages of the LST population in the FTA stayed at or below the 1996 mean (end of the treatment period) for the next three years; ivermectin treatment provided management of a LST population at least two years after treatment ended.

Three situations that occurred over the six-year treatment program stress the importance of complementing rate estimates with mean separation as tools in determining impact of ivermectin on the LST population. First, treatment means during the actual treatment did not differ across the three-year period for the FTA or NTA for female LSTs (Fig 3.2). However, the rate analysis was significant. The important consideration is the size of the means relative to the NonTA. When taking this relative size and the significant rate estimate into account, it is obvious that the treatment area means are shrinking while NonTA means remain the same. Secondly, in 1999 the male and

nymphal LST populations in the NonTA rose significantly (Fig 2.3 and 2.4, respectively). Also, the female population appeared close to significant graphically (Fig. 2.2). The populations in the FTA and NTA means for nymphs also were significantly higher than the previous year; males and females were non-significant, but appeared to be close graphically. At first glance of the graphical nymphal data in the NTA, it appears that management has not occurred in the NTA. However, the rate estimate of 1997 to 1999 nymphal data is significant and comparing the nymphal means in the NTA to the NonTA makes it clear why. This phenomenon is probably due to a mild 1998 to 1999 winter where conditions were favorable for more overwintering ticks to survive. Finally, it appears obvious that the larval population decreased significantly in the NTA from 1997 to 1998 relative to the NonTA. However, in this case, the rate estimate does not differ. This lack of difference is probably due to the fact that NTA and NonTa means did not differ significantly in 1997 (Fig 2.5). Since the 1994 to 1996 rate analysis was significant, the 1997 to 1999 rate analysis of larval LST data will almost certainly be significant once 1999 data are available.

The results of the analysis of sampling methods provide useful information to those interested in LST population estimates. Most adults were captured in the woods in the spring months by means of dry ice traps (Fig. 2.6 and 2.7). However, most nymphs were captured in the woods in the summer and fall months with cloth flags (Fig. 2.8). The habitat occurrence and seasonal distribution of the two stages are in accordance with Hair & Howell (1970) and are not surprising information. However, the relative success of the two sampling methods in capturing nymphal LSTs directly contrasts with the

results of Kinzer et al. (1990) who performed a similar study in Mississippi and Davidson et al. (1994a) in Georgia. In the Mississippi study, more nymphs and even larvae were captured with CO_2 traps. The traps in Mississippi sublimated for six hours (Kinzer et al. 1990). The traps in Georgia sublimated for one hour (Davidson et al. 1994a), the same length of time as the current study, which suggests geographic variability in sampling success regardless of the length of CO_2 trap sublimation. These results indicate that use of either CO_2 traps or cloth flags to sample LST populations, but not both, may lead to inaccurate estimates of the population. In addition, the cloth flags are absolutely necessary for sampling larval masses when sampling for periods of one hour. From 1994 to 1996, only one larval mass was captured with CO_2 traps, which is the reason CO_2 trapping was not continued in the 1997 to 1999 treatment after August of each year when the other two stages stopped responding to the CO_2.

ii. Adjacent Habitat Distribution of Lone Star Ticks

The results of the LST adjacent habitat distribution are straightforward. It is apparent that the most likely place one will encounter the LST is in the woods. Several adult ticks were found in the ecotone area as well. This information is valuable; even approaching wooded areas can be hazardous in terms of acquiring LSTs potentially harboring disease-causing agents. Particularly in areas like FFG where the HME outbreak occurred, golfers who knock a ball into a wooded area should either wear protective clothing when retrieving it or drop a new ball. A lower score is not worth

placing oneself at risk of acquiring HME. All outdoor enthusiasts should wear protective clothing and repellent when entering wooded areas.

The observation of large concentrations of nymphal LSTs in blueberry patches warrants further research, because vegetation was not quantified in the sampling areas. Quantification of deer sign could potentially test the hypothesis that nymphs are in blueberry as a consequence of deer bedding in the patches when larvae drop off. However, the large range of deer would probably require much larger sampling plots than the ones in this study to obtain reliable data.

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