To the Graduate Council:

I am submitting herewith a thesis written by Kimberly R. Hutchison entitled "Use of Ivermectin-treated Baits for the Management of the Lone Star Tick, *Amblyomma americanum* L. (Acari:Ixodidae)." I have examined the final 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.

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USE OF IVERMECTIN-TREATED BAITS FOR MANAGEMENT OF THE LONE STAR TICK, *Amblyomma americanum* L. (Acari:Ixodidae)

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ABSTRACT

Ivermectin-treated corn was fed to deer from March to August 1994 and 1995 in a tick-infested area of Fairfield Glade, Cumberland County, Tennessee. All life stages of *Amblyomma americanum* L. were collected from an ivermectin-treated and a non-treated area in 1994 and 1995.

In 1994, 3.4 times as many adult ticks were collected in the treated area as compared to the non-treated area. Approximately 2 times as many nymphs and 1.6 times as many larval masses were collected in the non-treated area. Adults were 1.7 times and nymphs were 1.5 times more numerous in the treated area as compared to the non-treated area in 1995.

Statistical analysis revealed that ivermectin treatment had no effect on the densities of lone star ticks in the treated area in 1994 and 1995. However, there was a reduction in the numbers of all life stages collected in the treated area in 1995 compared to 1994. Also, the number of larval masses collected in the treated area in 1994 was 4 times less than what would have been expected when compared to the high numbers of females collected earlier in the season and the number of masses collected in the non-treated area. Though no significant reductions were found, ivermectin may be causing a slow reduction in free-living tick populations in the treated area. An extended study and

treatment schedule could further reveal the effects of ivermectin treatment.

Seasonal distributions in 1994 and 1995 are presented for the life stages of *A. americanum*, based on numbers of individuals collected in the non-treated area. Differences in apparent seasonal densities due to sampling method were found for nymphal and adult ticks.

Significantly more nymphs were collected from the wooded areas than the grassy areas. No significant differences were found in the numbers of females and larval masses collected in the wooded and grassy areas.

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CHAPTER I

INTRODUCTION

Ticks have long been known to cause irritation and annoyance to humans, livestock, and pets as well as being important disease organism vectors. Greek writers, such as Homer and Cato, documented tick feeding patterns and their effect on humans and animals. Ticks are second only to mosquitoes as vectors of life-threatening disease organisms and, more importantly, transmit a larger assortment of pathogens than any other arthropod group. Tick bites are often painful and may cause not only disease infection but allergic and toxic reactions, paralysis, and economic losses due to blood loss. Ticks also reduce recreational values of parks and camping sites (Sonenshine 1993).

Review of Tick Control

Control of ticks and tick-borne diseases are concerns in many areas in the world, including the United States. Problems faced when attempting to manage tick populations include: broad dispersal of ticks throughout vegetation, fixed feeding sites of the tick on its host, concealed habits and hiding spots, huge reproductive capacity, dispersal on wild and domestic hosts, and longevity. The goal of most tick control programs is to reduce or eliminate

the pathogens transmitted by ticks and reduce the numbers of ticks attacking humans or animals to a desired level of management at a reasonable cost (Sonenshine 1993). Some current and former methods of tick control include chemical acaricides, habitat modification, host manipulation and/or eradication, biological control, self-medication, and personal protection.

Chemical Acaracides

During the early nineteenth century, arsenic-based acaricides were used for tick control on livestock. Arsenic dips were developed in the 1910s and were used until the 1960s, when chlorinated hydrocarbons such as dicholordiphenyltrichloroethane (DDT) became popular and considerably less expensive. (Ransom & Graybill 1912, Graybill 1913). DDT was used on a large scale after World War II, but it, along with many other chlorinated hydrocarbons such as lindane, aldrin, and dieldrin, were banned from use in the 1970s (Sonenshine 1993). Toxaphene, another chlorinated hydrocarbon, was used against ticks until its ban in the 1980s (Matsumura 1985, Brown 1978).

Three classes of chemical pesticides currently used for tick control include organophosphates (coumaphos and diazinon), pyrethrins (permethrin and flumethrin), and carbamates (carbaryl and propoxur) (Sonenshine 1993, Drummond et al. 1988). Both organophosphates and carbamates

inhibit acetylcholinesterase, causing nervous system inhibition, whereas pyrethrins are fast acting acaricides with rapid knockdown. Pyrethrins affect synapses causing death or paralysis and also may work as repellents (Brown 1978).

A recently discovered pesticide, ivermectin, has proven effective for control of many pests, including insects, nematodes, and ticks. Ivermectin is a derivative of avermectins, a family of natural macrocyclic lactones derived from *Streptomyces avermitilis* (Lasota & Dybas 1991, Campbell et al. 1983). The acaricidal activity of ivermectin has been demonstrated against *Boophilus microplus* (Canestrini), *Amblyomma americanum* L., *Dermacentor variabilis* (Say), and *Ornithodoros parkeri* Cooley (Pound et al. 1995, Ash & Oliver 1989, Wilson et al. 1991). Though the exact mode of action is unknown in ticks, ivermectin generally interferes with neurotransmission via the gamma-aminobutyric acid route. Ivermectin causes chloride gated channels to remain open, blocking nerve action and muscle response to stimuli (Lasota & Dybas 1991, Ash & Oliver 1989). Ivermectin also is known to disrupt engorgement, molting, egg production, chitin synthesis, oviposition, and to cause paralysis and eventually death (Lunke & Kaufman 1992, Kaufman et al. 1986, Wilson et al. 1991).

Acaricide application techniques vary in their advantages and targets. Direct whole body treatment,

systemics, and controlled delivery systems are used to treat domestic animals such as livestock and companion animals.

Practical control of *Boophilus annulatus* (Say) and *B. microplus* began in the United States in the early 1900s in Texas to eradicate Texas Cattle Fever. This control program led to the development of the cattle dip, a method of immersing animals in water treated with an acaricide. Because *B. annulatus* and *B. microplus* had only recently been introduced and their hosts were limited primarily to cattle, this method was effective in eliminating Texas Cattle Fever and its vectors (George 1989). Dips became the primary method of control for the first half of the twentieth century. Today dipping also is used for small domesticated animals, but acaricide-containing shampoos have replaced dips for home treatment of dogs.

Application of pesticide emulsions or suspensions was introduced on a broad scale after World War II. Pyrethroids, such as lambdachalothrin, are the most popular emulsions for tick control on livestock and pets (Davey et al. 1992). The use of sprays has become more prevalent than dips due to lower capital investments and animal stress. However convenient, spraying cannot assure complete animal coverage. Factors such as wind, animal movement, and operator skills cause variations in extent of coverage. Pesticide waste is often considerable due to uncontrollable runoff (Sonenshine 1993).

Non-systemic pour-ons are a more recent form of acaricide delivery. A liquid containing a dissolved toxicant, such as flumethrin, deltamethrin, or permethrin is applied along the spinal area of livestock (Corn et al. 1994, Stendel 1985). Toxicants are dispersed by the inert carrier over the animal's body. Advantages of pour-on use include ease of delivery, pesticide volume reduction, and waste reduction, but treatment may be less thorough than dips or sprays.

Dusts consist of a pesticide mixed with a powder such as talc and are applied to the animal's body. Small hand-held containers are available for home treatment of pets. Dust bags are used for livestock, providing a passive system of self treatment. Livestock pass under or near the bags, causing the pesticide to be applied to the animals spinal area.

Backrubbers work in much the same way for cattle treatment. Burlap treated with an oil solution of pesticide is wrapped around a cable or chain suspended between two posts. Animals rubbing against the cloth spread pesticide over their bodies (Drummond et al. 1988).

Systemic insecticides are used to treat an animal's entire system. Insecticides are introduced into the animal via treated food, mineral supplement, or subcutaneous injection, or they may be absorbed through the skin after a pour-on treatment. Systemics have low vertebrate toxicity

but greatly affect target parasites, including ticks, that attempt to feed on the vertebrate. Pesticides enter the parasite's system along with the ingested blood meal. Ivermectin and benzimidazoline have proven to be particularly effective systemics for tick control (Drummond et al. 1988, Boisvenue & Hair 1985).

Sustained release of pesticides is one of the most common forms of tick control. Pet owners use tick collars made of acaricide-impregnated plastic for home tick control. Eartags, also commonly used for livestock, deliver pesticides, such as cypermethrin, tetrachlorvinphos, flucythrinate, propetamphos, or fluvalinate, to the ears, head, and neck; areas most likely to be tick-infested (Rechav 1987, Owen 1985, Taylor et al. 1984). Eartags have proven effective for control of the Gulf Coast tick, *A. maculatum* Koch and *B. microplus* (Drummond et al. 1988, Owen 1985).

Vegetation also may be treated with acaricides for management of tick populations. Pesticides are sprayed from airplanes or machine-powered sprayers and offer wide-spread coverage. Vegetation treatment has been used to control *B. annulatus* in the southern United States and *D. variabilis* in Long Island and Nova Scotia (Glasgow & Collins 1946). Spraying has been more recently used in Lyme disease control, and for *A. amblyomma* in recreational areas (Bloemer et al. 1990). However, effectiveness is limited by

inability to penetrate dense ground cover, adverse weather, and acaricide instabilities. Granular formulations work best for vegetation penetration though large quantities of pesticide are necessary to reach ticks in their habitat (Bloemer et al. 1990). Acaricides used for spraying include diazinon, chloropyrifos, and carbaryl. As public awareness increases, more concern for this type of widespread treatment and its adverse impact on the environment will develop.

Habitat Modification

An alternative to chemical acaricide use is habitat modification. This type of change in the natural environment of the tick utilizes no insecticide application.

The primary goal of habitat modification is to alter or destroy the microhabitat of the tick. This alteration or destruction may be accomplished by treatment with herbicides, controlled burning, mechanical clearing, or leaf litter removal (Davidson et al. 1994; Barnard et al. 1988, Presley & Hair 1988). These practices expose ticks to extreme desiccation, intense heat in the summer, and cold in the winter. Habitat modification also may reduce the number of hosts available for tick parasitism. The causes of tick mortality, whether indirect or direct, are unknown. Desiccation as well as starvation may play important roles.

Habitat modification is labor intensive and requires a high level of maintenance. Control lasts only for a limited time (Davidson et al. 1994).

Burning has been shown to reduce densities of *D. variabilis* and the winter tick, *D. albipictus* (Packard)(Smith et al. 1946, Drew et al. 1985). Management of the lone star tick in recreational areas has also been achieved with some success using burning and mechanical clearing (Clymer et al. 1970, Bloemer et al. 1990).

Host Eradication/Eradication

Host eradication was one of the earliest forms of tick-borne disease management. In 1911, thousands of Columbian ground squirrels, pine squirrels, chipmunks, and other rodent hosts of *D. andersoni* Stiles were eliminated by poison baits, trapping, or shooting to control Rocky Mountain spotted fever. This practice was continued for several decades. The area was quickly repopulated by hosts with ticks from non-treated areas. Ticks continued to be present, demonstrating that the method was both ineffective and expensive (Cooley 1932). A similar study was conducted in Martha's Vineyard, Massachusetts, to control *D. variabilis* by eradicating meadow voles. Vole and tick populations were reduced when compared to non-treated control areas; however, populations quickly rebounded after the program was terminated (Smith et al. 1946).

Eradication is successful if the intended control area is isolated and repopulation is limited. The success of eradication was demonstrated by Wilson and co-workers (1988) who eliminated a complete population of white-tailed deer in an island community on Great Island, off the Massachusetts coast, to assess the effect of host eradication on *Ixodes scapularis* Say. This study showed that eradication is effective but limited and slow. Densities of ticks were significantly reduced but still present in low numbers after three years. Eradication of this type would be complex on the mainland as deer eradication would be difficult to maintain in large areas of land.

Host exclusion, removal of hosts from specific tick-infested areas, is successful with livestock, where this process is known as pasture rotation or pasture spelling. Rotation is effective against the sheep tick, *I. ricinus* (L.), as well as *A. americanum*. In Oklahoma, densities of lone star ticks were reduced by 76% during a 12-year period using a rotation program and by 98% when livestock were totally excluded (Clymer et al. 1970).

Deer exclusion is only slightly less effective than acaricidal treatment or vegetative management for decreasing numbers of lone star ticks in the densely infested Land Between the Lakes area of Kentucky and Tennessee (Bloemer et al. 1990). Exclusion may be accomplished using high tension, electrified wires or deer-proof fences. Though this

procedure is initially expensive, its effectiveness lasts for many years and requires relatively little maintenance when compared to labor intensive acaricide application and vegetation management (Bloemer et al. 1990). However effective exclusion may be, *A. americanum* and other ticks, remain opportunistic and may attack other medium-size vertebrate hosts, such as raccoons, if deer are excluded (Zimmerman et al. 1987). Exclusion of these alternate hosts also reduces recreational and aesthetic value of the treated areas (Sonenshine 1993).

Biological Control

Biological control has not been often attempted with ticks. Few natural predators have been found that significantly reduce tick populations. One study evaluated a chalcid wasp, *Hunterellus hookeri* Howard, for control of *D. variabilis* and *I. scapularis* in eastern Massachusetts (Larrousse et al. 1928). Densities of *I. scapularis* and spirochete transmission remained high, despite the fact that more than 40% of the nymphal ticks were parasitized by the wasp. Parasitized ticks generally die after engorgement (Mather et al. 1987, Spielman 1988).

Ticks have been reported to be consumed by fire ants (*Solenopsis* spp.) and lycosid spiders. Both are effective predators that feed on engorged females or their eggs (Wilson & Deblinger 1993). Fire ants attack and consume

female lone star ticks as well as their eggs and larvae. Unfortunately, fire ants also attack many animals, including vertebrates and humans, making mass release impractical.

Plants that trap and kill larval ticks also may have biological control potential. Molasses grass (*Melinis minutiflora*) and two tropical legumes, *Stylosanthes hamata* and *S. scabra,* have been shown to trap and kill larva of the *B. microplus* (Drummond et al. 1988). The legumes, which often grow in pastures, have glandular hairs that secrete a viscous fluid. *B. microplus* are trapped by the secretions and are poisoned by an unidentified volatile compound in the plant matter. These secretions do not repel the larva, which walk into the material and become immobilized.

Self-medication

Self-application of acaricides to host animals is a method of tick control that utilizes host behavior to deliver acaricides in a highly discerning fashion. These techniques minimize the quantity of acaricide that is dispensed to the host as well as to the environment.

One of the first examples of self-medication was described by Sonenshine and Haines (1985). Polyvinyl chloride tubing covered with diazinon-impregnated oil (1%) were scattered into forest areas infested with *D. variabilis*. Tubes were baited with peanut butter to attract the tick's key hosts, mice and voles. Tick burdens in the

treatment area were reduced to 0.10-0.66 ticks/animal versus 3.51-4.50 ticks/animal in the control area. A control program of this type proposes extreme flexibility as baits and tube diameter can be varied to target specific tick hosts.

Another method of self-medication is the Damminix system, in which cotton fibers impregnated with acaricide are placed in host habitats (Mather et al. 1987). For example, white-footed mice use the cotton for nest building, delivering the acaricide to themselves and their nestmates. In a study by Mather and co-workers (1987), 76% of collected mice in the treated area were tick-free, whereas all the mice were tick-infested in the control area (Mather et al. 1987). Another study conducted by Daniels and co-workers (1991) found no significant differences in tick collections between areas treated with Damminix-impregnated cotton and control areas (Daniels et al. 1991).

Personal Protection

Awareness is the single most important protection for people planning to enter tick-infested areas. The more knowledge people have about ticks and tick-borne diseases, the more likely they will be to practice personal preventive measures such as: 1) wearing boots or securable footwear, 2) using repellents and/or acaricides on clothing and exposed skin, 3) tucking pant legs into boots, pulling socks over

pant cuffs, and sealing exposed socks with tape, and 4) examining skin and clothing at the earliest opportunity after exiting infested areas (Sonenshine 1993).

The Lone Star Tick

The lone star tick, *Amblyomma americanum* (Acari: Ixodidae), is one of the most important man-biting species of ticks in the southeastern United States (Sonenshine 1993). The lone star tick is an ectoparasitic pest of humans, wildlife, companion animals, and livestock (Barnard et al. 1988, Bolte et al. 1970).

Lone star ticks are found throughout the southcentral and southeastern United States and are particularly abundant in recreational areas supporting high densities of white-tailed deer (Barnard et al. 1988, Bolte et al. 1970). Lone star ticks are three-host ticks, and all three stages may be parasitic on large mammals (deer and cattle) as well as humans, birds, and medium-sized mammals (foxes, dogs, coyotes, and skunks) (Bishopp & Trembley 1945, Clymer et al. 1970, Patrick & Hair 1977, Zimmerman et al. 1987).

At least one of the lone star tick's parasitic stages feed on deer from April to October in Oklahoma (Patrick & Hair 1977), and February to October in western Kentucky and northwestern Tennessee (Land Between the Lakes) (Cooney & Burgdorfer 1974). Humans are subject to lone star tick

attack between March and October, particularly in the spring and early summer (Barnard et al. 1988).

Free-living adult ticks are found primarily between February and June in Land Between the Lakes (Cooney & Burgdorfer 1974) and March and July in central Georgia (Davidson et al. 1994). Adult lone star ticks attach within two to three hours after a suitable host is located. Males seek attached females for mating and attach beneath the female before mating (Berger et al. 1971). A single male tick can detach, find other females and fertilize as many as 36 females in 43 to 127 days (Gladney & Drummond 1970). Males feed on a host imbibing less than 0.003 ml of blood whereas females may imbibe 0.74 ml from deer (Sauer & Hair 1972). Males remain on the host until death. Females must be fertilized before engorgement can be completed, whereafter they must detach to oviposit (Barnard et al. 1988).

Oviposition takes place on the ground within five to ten days after the female detaches. Females oviposit for approximately 25 days, converting 60% of their engorged mass to eggs. About 450 to 500 eggs are produced each day, and a single large mass is deposited at the anterior end of the female's body (Drummond et al. 1971, Barnard et al. 1988).

Free-living larvae are found primarily between July and September in central Georgia and between July and October in Land Between the Lakes (Davidson et al. 1994, Cooney &

Burgdorfer 1974). Newly-hatched, six-legged larvae stay near the egg mass for 10 to 30 days (Patrick & Hair 1979). Larvae climb vegetation where they wait for passing hosts. Larvae that successfully encounter deer usually attach to the head and neck area of deer, particularly the ears, due to heavy vascularization, reduced hair covering, and protection from grooming (Bloemer et al. 1988). Larval engorgement lasts three to ten days during which larva imbibe 0.001 ml of host blood (Sactor et al. 1948, Koch 1986). Larvae then detach, overwinter, and emerge as eight-legged nymphs the following spring (Koch 1986).

Eight-legged, free-living nymphs are active one to twelve days after molting from larva to nymph and are found beginning in April. Nymphal activity occurs in August in Land Between the Lakes (Cooney & Burgdorfer 1974) and from March to September in central Georgia (Davidson et al. 1994, Robertson et al. 1975). Nymphs also climb vegetation to seek hosts, but, unlike larvae, nymphs are able to actively quest and move quickly across surfaces and are attracted to host-emitted carbon dioxide (Koch & McNew 1982). Nymphs, like larvae, attach to the head and neck of deer and engorge for 3 to 10 days (Koch 1982, Sactor et al. 1948). Nymphs detach, overwinter, and molt to adults in late winter/early spring.

Adult free-living lone star ticks are active 8 to 15 days after molting from the nymphal stage. They seek hosts

by climbing vegetation on warm spring days and questing for host emitted carbon dioxide or heat. Adults are able to travel on the ground at a rate of 9 to 15 meters per hour (Koch & McNew 1982).

The lone star tick is known to vector several disease causing organisms to both animals and humans. Humans may contract tularemia and possibly ehrlichiosis via tick bites (Hopla 1974, Olsen 1992). Spotted fever group rickettsiae also have been isolated from lone star ticks, which are suspected in the transmission of Rocky Mountain spotted fever, particularly in areas where *D. variabilis* is rare (Clifford et al. 1969, Anderson et al. 1986). However, with the exception of Parker and co-workers (1943), natural transmission of Rocky Mountain spotted fever with virulent *Rickettsia rickettsii* has not been proven. White-tailed deer may be infected with *Theileria cervi* via lone star tick and are believed to be the reservoir of *Ehrlichia chaffeensis*, a rickettsia that causes human ehrlichiosis (Kocan & Kocan 1991, Sonenshine 1993).

Ehrlichiosis

Human illness caused by an *Ehrlichia* spp. was first recognized in the United States in 1986 (Anonymous 1990). Originally believed to be caused by *Ehrlichia canis*, a canine leukotropic rickettsia, it was later identified as a

separate, similar organism, *E. chaffeensis* (Anderson et al. 1991, 1992). Since its recognition in 1986, 220 cases have been diagnosed in 20 states in the United States (Dawson et al. 1991). Human ehrlichiosis causes several flu-like symptoms, including fever, headache, nausea, malaise, and gastrointestinal symptoms (Standaert et al. 1995). Most patients have a history of tick exposure three weeks prior to illness and reside in rural areas. Though the exact vector of *E. chaffeensis* is still unknown, most epidemiological evidence suggests *A. americanum*. The vertebrate reservoirs also are unknown but serum studies indicate the white-tailed deer (Olsen 1992, Standaert et al. 1995). *A. americanum* in areas with high population densities of white-tailed deer support this relationship (Patrick & Hair 1977).

Research objectives of study

Researchers at the United States Department of Agriculture Livestock Insect Research Laboratory, Kerrville, Texas, have conducted several studies within controlled environments dealing with ivermectin control of lone star ticks on white-tailed deer. One of these studies utilizes a deer-feeding technique to target all three of the parasitic stages of the lone star tick. The United States Department of Agriculture's studies have shown that feeding ivermectin-

treated corn to deer reduces the number of free-living lone star ticks in treatment areas (Pound et al. 1995). Most of the ticks that feed on deer that have consumed ivermectin-treated corn will die in place without detaching. Of those that do detach, males develop normally but females have reduced ovaries with few oocytes, resulting in fewer eggs and reduced hatching. Most nymphs and larvae will die in place.

During the summer of 1993, the resort area of Fairfield Glade, Cumberland County, Tennessee, experienced an outbreak of human ehrlichiosis. This disease has a normal incidence rate of three to five cases per 100,000 people. Fairfield Glade had ten from a population of 4,000 people. Four of these ten cases had visited a doctor due to the severeness of their symptoms. Serological tests of randomly selected residents indicated that as high as 12% of the population of Fairfield Glade had been exposed to *E. chaffeensis*, with most cases being asymptomatic (Standaert et al. 1995).

Ticks collected during the summer of 1993 were primarily lone star ticks. Due to the high populations of deer in the area and the presence of lone star ticks, it is feasible to hypothesize that controlling the numbers of free-living lone star ticks may reduce the incidence of *E. chaffeensis*. Application of the United States Department of Agriculture's ivermectin delivery technique to an area such as Fairfield Glade could disrupt the reproductive cycle of

the ticks, reduce their free-living numbers and thus the transmission of *E. chaffeensis*.

The objectives of this two year study were to: 1) determine if feeding ivermectin-treated baits to white-tailed deer reduces free-living lone star tick populations 2) determine the seasonal abundance of each of the lone star tick's developmental stages, and 3) compare the populations of lone star ticks in wooded areas and mowed roadside areas.

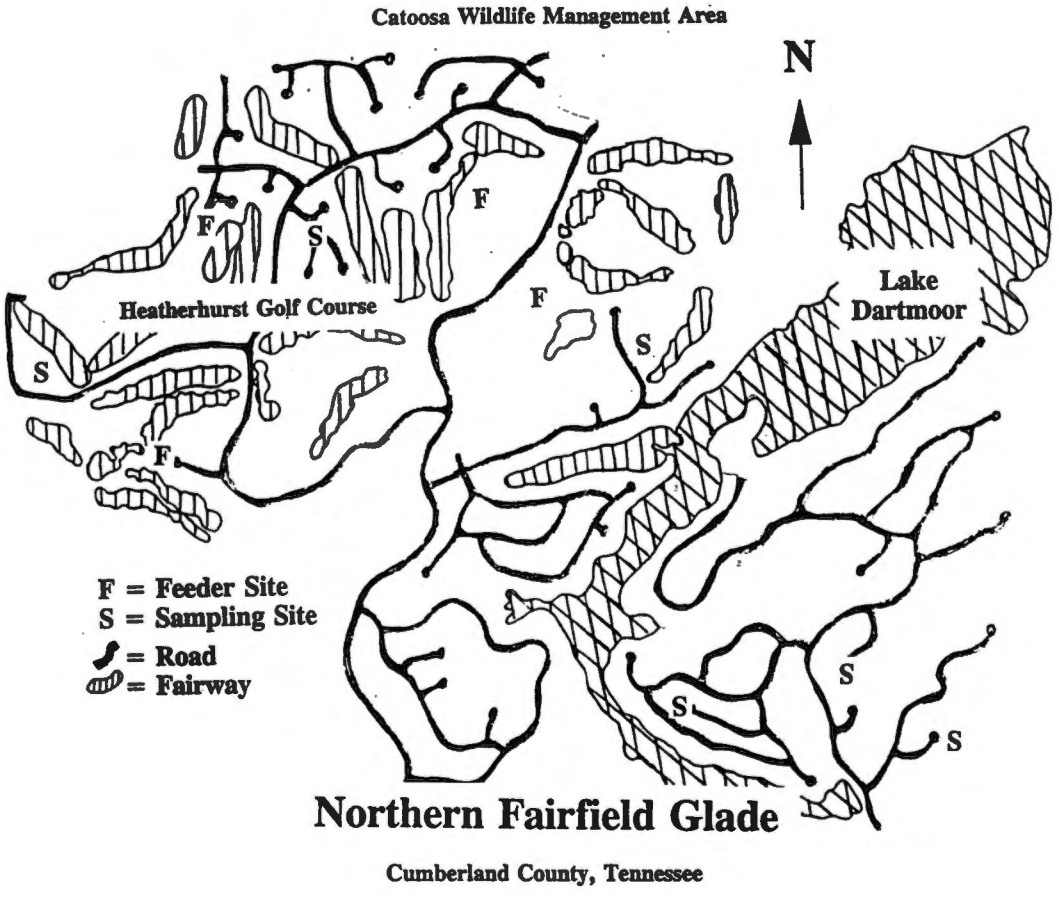


Fig. 1. Study site, northern Fairfield Glade, Cumberland County, Tennessee.

CHAPTER II

MATERIALS AND METHODS

Field research was conducted within the boundaries of Fairfield Glade in Cumberland County, Tennessee. Fairfield Glade, a retirement community of approximately 4,000 residents, lies on the Cumberland Plateau of eastern Tennessee and is bounded on the north by Catoosa Wildlife Management Area. This recent resort community, whose development began in 1970, consists of home sites, golf courses, forests, lakes, roads, and several recreational park areas. This community is built in a second-growth mixed mesophilic forest with an average elevation of 600m.

The forest overstory of Fairfield Glade is primarily composed of mixed hardwoods and pines, including sourwood (*Oxydendrum arboreum* L.), white pine (*Pinus strobus* L.), white oak (*Quercus alba* L.), hickory (*Carya sp.*), and black gum (*Nyssa sylvatica* L.). The understory also includes white pine and white oak, as well as blueberry (*Vaccinum sp.*), holly (*Ilex sp.*), and red maple (*Acer rubrum* L.).

Research Area

Research was conducted in the northern regions of Fairfield Glade, adjoining Catoosa Wildlife Management Area (Fig. 1). The treated area (approximately 445 ha) was located on the west side of Lake Dartmoor and surrounds

the Heatherhurst Golf Course. The area is composed of rolling hills with golf courses, home sites, and mixed hardwood/pine forests.

The non-treated area is separated from the treated area by Lake Dartmoor. The non-treated area is located on the east side of Lake Dartmoor and encompasses an area approximately 445 ha. This area is topographically comparable to the treated area with the only difference being the lack of a golf course. The forests are mixed hardwood/pine.

Deer Feeders

Recleaned corn (livestock feed corn that had been cleaned twice with air pressure) was dispensed in the treated area from four battery powered, solar charged, automated deer feeders (Specialty Systems, Inc. Austin, Texas). Corn stored in the feeder barrel reservoir was dispersed onto the surrounding ground by a rotating motor. Timers permitted adjustment for changing feeding times, daylight hours, and dispersion duration. Feeders were programmed to dispense corn once before daylight and once at dusk and were placed in cleared areas with high deer usage and/or populations, as well as away from golfers and roads (Fig. 1). Each feeder held approximately 136 kg of corn and was refilled as needed. Feeders and corn were not used in the non-treated area.

Observations of squirrels using the feeders during 1994 led to the need for better control of medicated corn consumption. Squirrel guards were added to the feeders in 1995. Bands of chicken wire, 2.5cm mesh, approximately 0.5m wide, were placed around the bottom of the feeder funnels to prevent squirrels from manipulating them.

Feeders began to dispense non-treated, recleaned, whole kernel corn on 11 March 1994 and 6 February 1995. Non-treated corn was dispensed for approximately one month in 1994 and 1995 to adjust deer to the presence and noise of the feeders as well as to establish a feeding schedule.

Treated corn was dispensed beginning on 8 April 1994 and 13 March 1995 (Table 1). Feeders were monitored approximately every two weeks to assess usage of corn by deer and refilled as needed. Estimates of deer usage in the treated area were based on the relative amount of corn left on the ground by deer during the daylight hours after the early morning feeding. Treated corn was dispensed until the end of July in 1994 and 1995 allowing 90 days for ivermectin to leave the blood stream of treated deer before the start of hunting season.

Corn Treatment

Each 22.7 kg of whole corn was treated with 50 ml of Ivomec® Pour-On solution, a pour-on treatment for cattle containing 5mg ivermectin/ml. Each 22.7 kg of corn and 50

Table 1. Amounts of treated and non-treated corn dispensed in the treated area in 1994 and 1995.

Year	Feeder No.	Non-treated Corn (Kg)	Treated Corn (Kg)
1994	1	136.2	331.4
	2	136.2	408.6
	3	136.2	442.7
	4	136.2	429.0
1995	1	136.2	567.5
	2	136.2	454.0
	3	136.2	590.2
	4	136.2	567.5

ml of Ivomec® were allowed to mix for 3 minutes in a small concrete mixer. Deer consumption of 0.45 kg of treated corn per day delivers an acaricidal dosage of 35-50 μg of ivermectin per kg of deer weight (Pound et al. 1995).

Sampling Sites

Three tick sampling sites were selected from both the treated and non-treated areas (Fig. 1). Random collections were made three times at numerous sites chosen using flannel drags and carbon dioxide traps (See procedure below). Site selection was based on topographical similarities between the treated and non-treated areas and consistency of tick populations as determined by the pre-study collections. Selected sites were comprised of both forest and mown grassy roadside areas and had similar plant and geographical features, as well as deer usage.

Lone Star Tick Collections

Adult and nymphal lone star ticks were collected with carbon dioxide traps and flannel tick drags (Grothaus et al. 1976). Two carbon dioxide traps were placed in each treated and non-treated sampling site. One trap was placed in a wooded section of the site and another in a grassy section. A carbon dioxide trap consisted of a block of dry ice approximately 0.5-1.0 kg placed on one m^2 of white nylon cloth. Four, 1.15 mils, sealable plastic bags were filled

with sand and used to hold down the corners of the cloth. Traps were placed in the sampling sites between the hours of 9:00 AM and 3:00 PM Central Standard Time so they could be operated during periods of maximum daytime temperatures and tick activity. After one hour, adult and nymphal ticks were counted and recorded according to sex from the top and bottom of the cloth.

Adults, nymphs, and larvae were collected using flannel tick drags. One m^2 of white flannel attached to wooden dowels was dragged 10m, ten times at each sampling site (Grothaus et al. 1976). As with the carbon dioxide traps, drags were made in the woods and along the grassy roadside for a total of two sets per sampling site. Drag sampling was increased to 20 ten m drags per site between 24 August and 19 September 1994 to more closely monitor larval mass numbers. Adults and nymphs were removed from drags and carbon dioxide trap cloths by hand, counted, returned to the ground of the collection site, and recorded as total numbers of each per 100 m of drag. Due to the minute size of larval ticks, larval masses were counted rather than individuals.

Biweekly collections were made at different places within the sampling sites to avoid sampling bias. Counts were made at approximately two-week intervals from March to October, 1994, and March to July in 1995.

Data Analysis

Tick collection data for 1994 and 1995 were paired by date proximity and were transformed using $\sqrt{(X + 0.5)}$ transformation. Data were analyzed with a repeated measures general linear model (GLM) at $P < 0.05$ and Duncan's Multiple Range Test (SAS Institute 1989). Treatment, year, collection dates, sampling sites, and sampling type (grass or woods) were considered main effects.

CHAPTER III

RESULTS AND DISCUSSION

Tick Collections

1994. Adult, nymphal, and larval lone star ticks were collected from treated and non-treated areas using carbon dioxide and drag sampling methods. More than 3.4 times as many adult ticks were collected in the treated area than in the non-treated area (Fig. 2 and 3). Approximately two times more nymphs were collected in the non-treated area than in the treated area (Fig. 4 and 5). More than 1.6 times as many larval masses were collected in the non-treated area than in the treated area (Fig. 6).

1995. Collections of adult and nymphal ticks with carbon dioxide and drags in yielded 1.7 times more adults in the treated area as compared to the non-treated area (Fig. 2 and 3). Nymphs in the treated area were 1.5 times more numerous when compared to those in the non-treated area (Fig. 4 and 5). As larval tick masses do not appear until July through September, larval ticks were not included in this study for 1995. However they will be collected and included in future analysis.

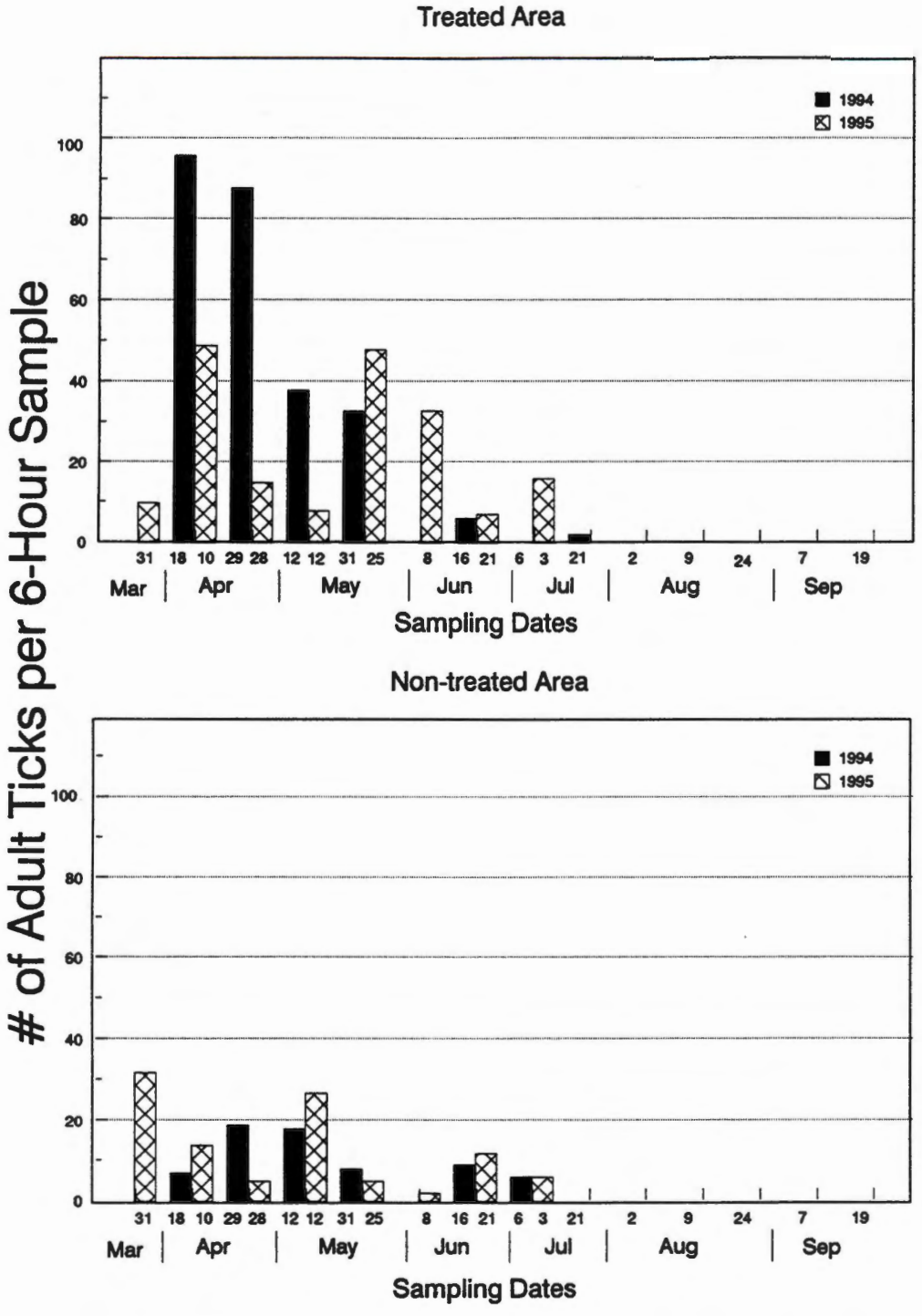


Fig. 2. Adult lone star ticks collected by carbon dioxide sampling in the treated and non-treated areas in 1994 and 1995.

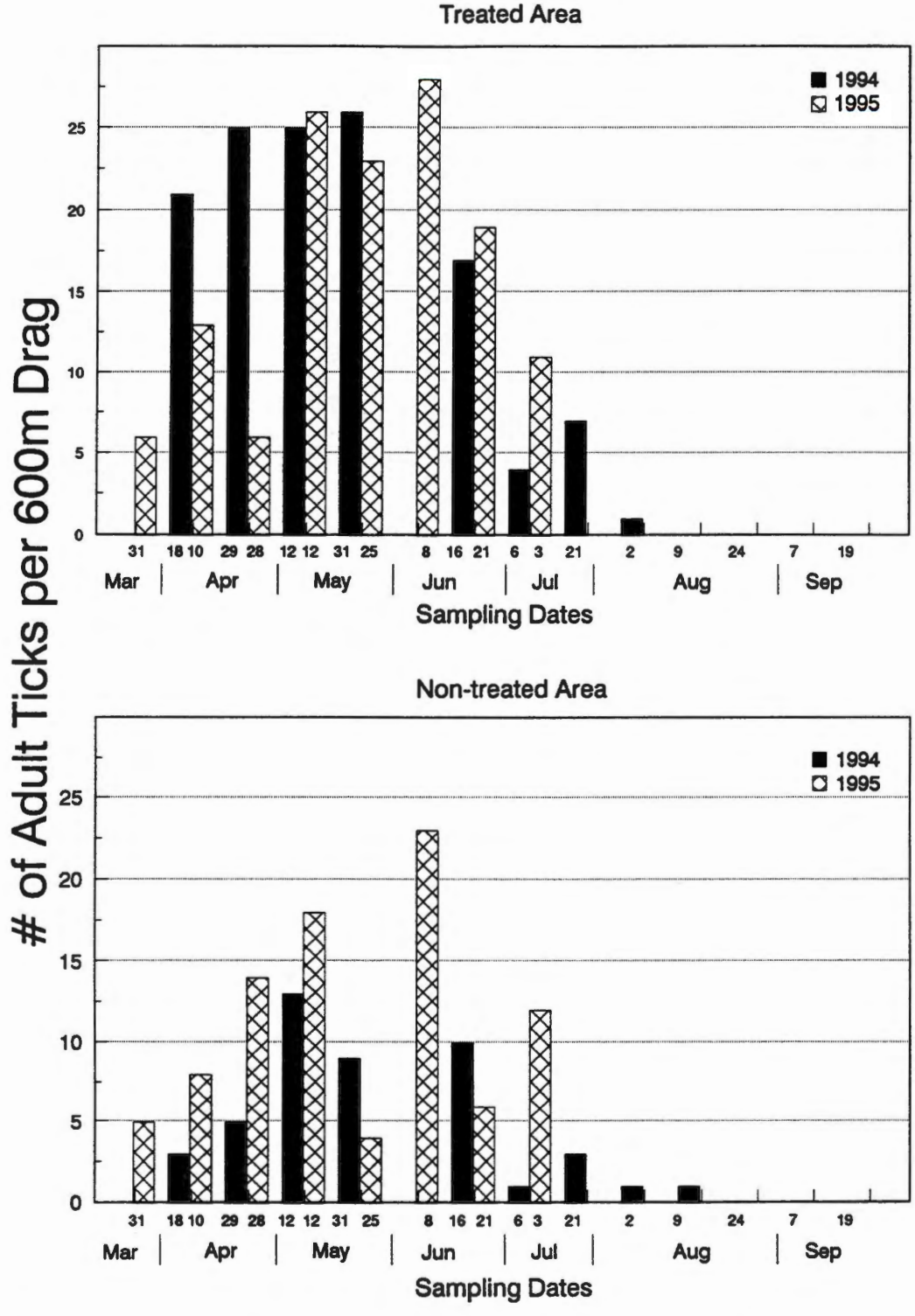


Fig. 3. Adult lone star ticks collected by drag sampling in the treated and non-treated areas in 1994 and 1995.

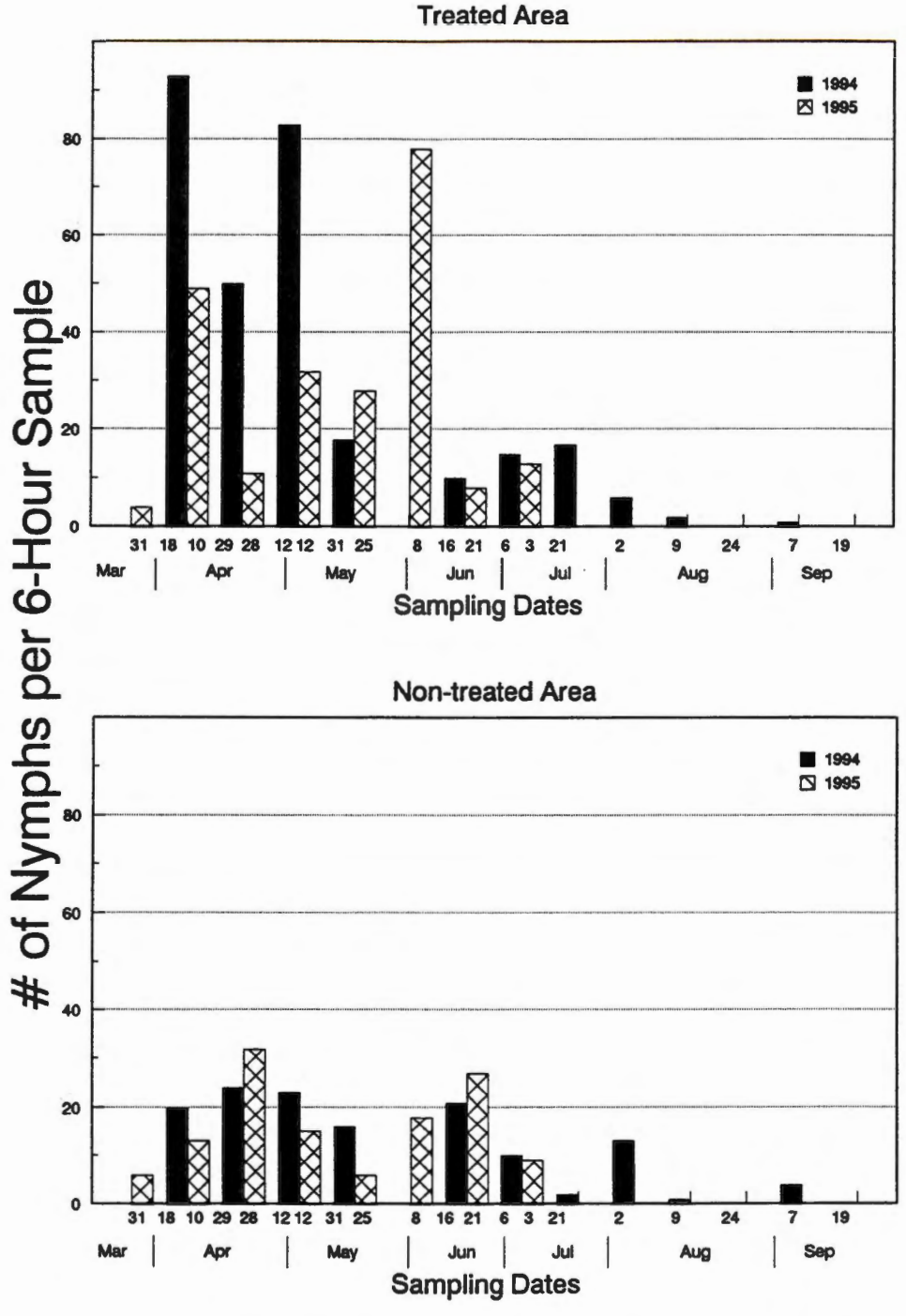


Fig. 4. Nymphal lone star ticks collected by carbon dioxide sampling in the treated and non-treated areas in 1994 and 1995.

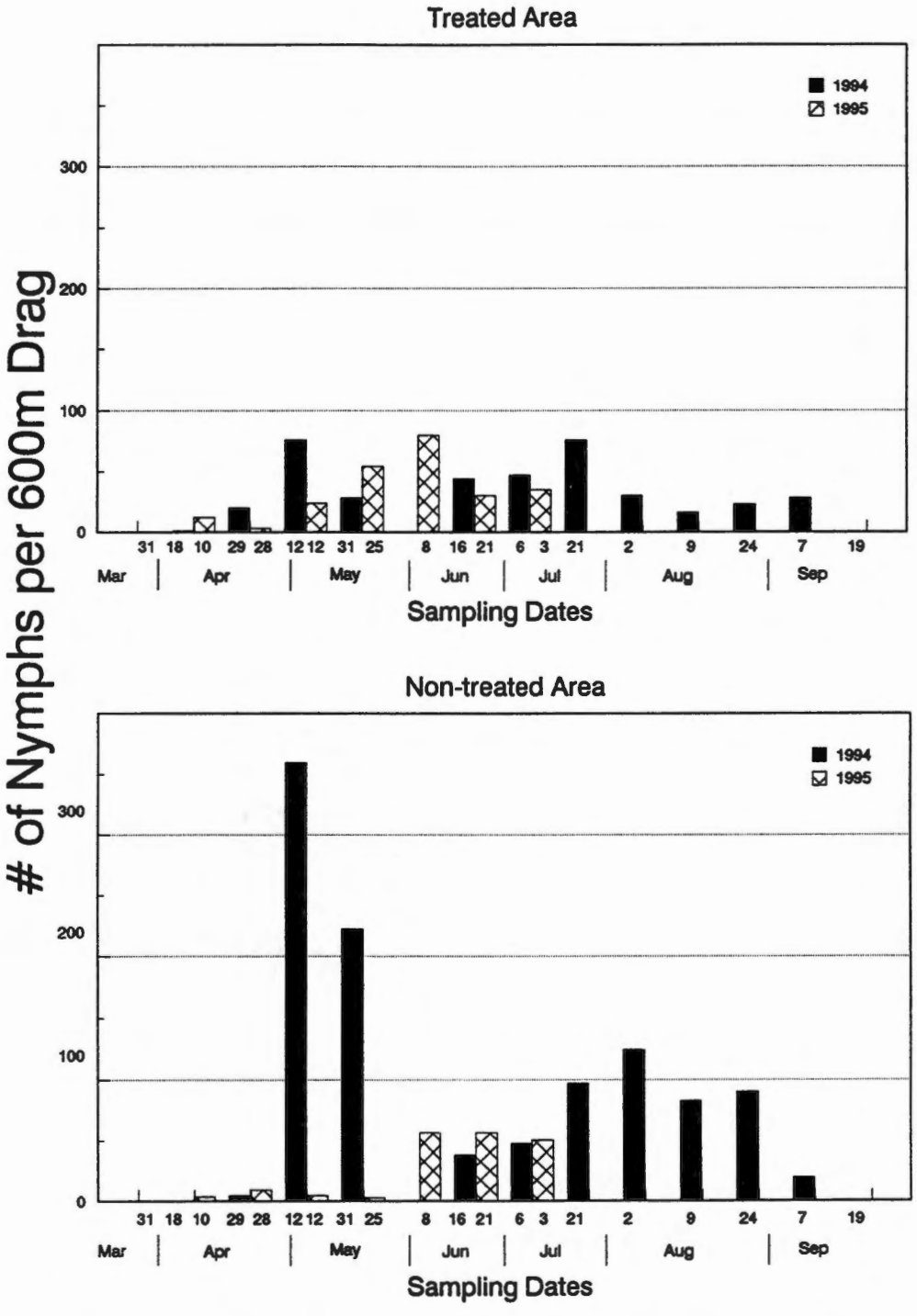


Fig. 5. Nymphal lone star ticks collected by drag sampling in the treated and non-treated areas in 1994 and 1995.

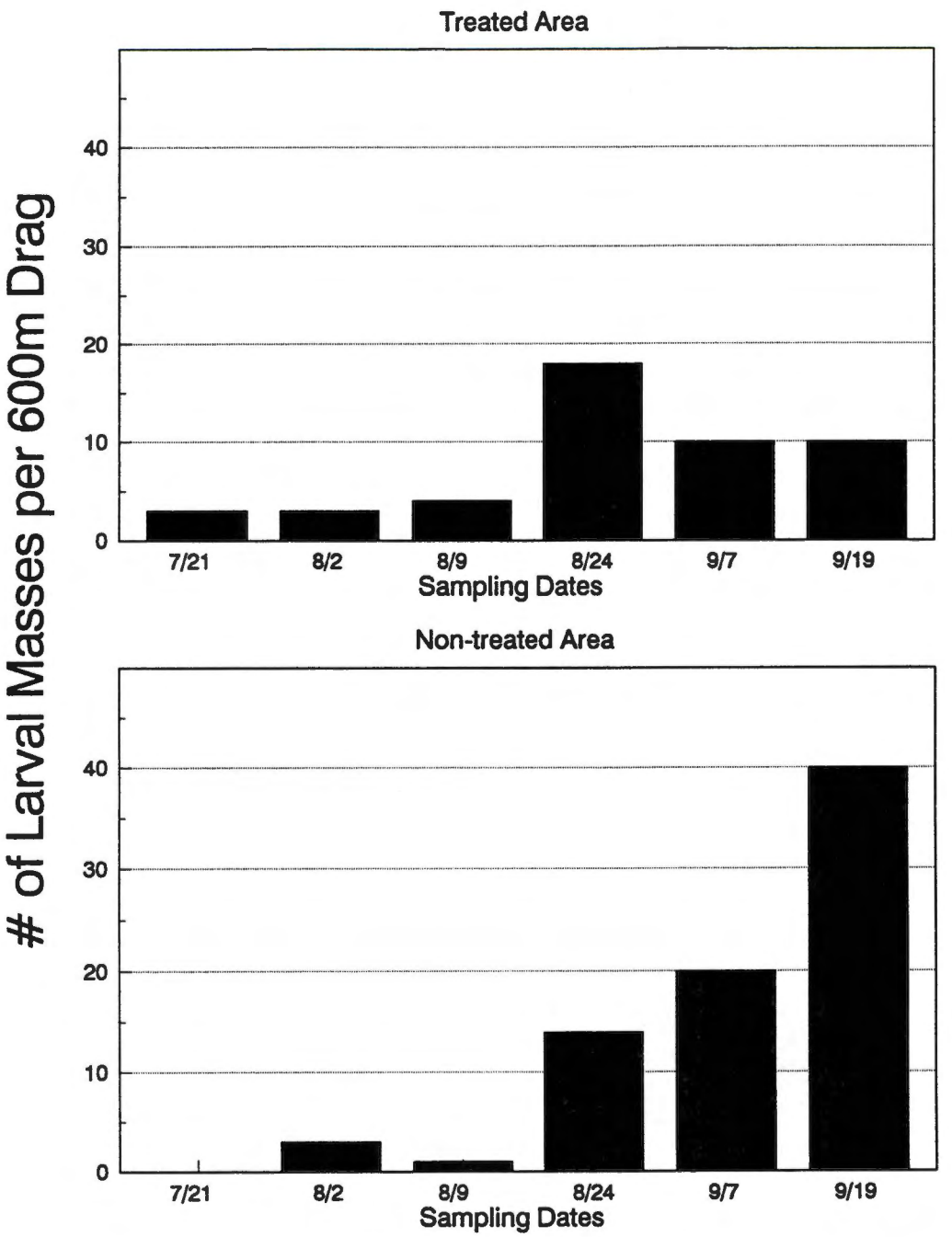


Fig. 6. Lone star tick larval masses collected by drag sampling in the treated and non-treated areas in 1994.

Impact of Ivermectin on Free-living Lone Star Ticks

Significant differences were detected between densities of lone star ticks in treated and non-treated areas and between years, 1994 and 1995. In 1994, significantly more females were collected in the treated area than the non-treated area. (Table 2). Due to their life cycle, ticks in the treated area had not yet fed on ivermectin treated deer. The number of nymphs collected in the treated area and the non-treated area were not significantly different (Table 2). Total ticks (adults plus nymphs) collected in 1994 were not significantly different between treated and non-treated areas (Table 2). Densities of collected larval masses were not significantly different between the treated and non-treated areas (Table 2). This non-significance may reflect the influence of ivermectin treated as the significantly larger population of females in the treated area should have produced significantly larger numbers of larval masses.

Densities of females collected in 1995 were significantly different due to treatment. Significantly more females were collected in the treated area than the non-treated area (Table 3). Nymphs collected in 1995 were also significantly different due to treatment, with more nymphs and total ticks collected in the treated area (Table 3). Larval masses had not yet been collected by 3 July, 1995.

Table 2. Influence of ivermectin treatment on densities of lone star ticks life stages in 1994.

Treatment	Mean/Sampling Day ± SD[1]		n	Stage
ivermectin-treated	1.62 ± 4.11	a	144	female
non-treated	0.41 ± 0.99	b	142	female
ivermectin-treated	4.21 ± 7.26	a	144	nymph
non-treated	9.40 ± 35.41	a	142	nymph
ivermectin-treated	7.00 ± 9.27	a	144	total ticks[2]
non-treated	10.01 ± 35.41	a	142	total ticks
ivermectin-treated	0.36 ± 1.10	a	144	larval mass[3]
non-treated	0.47 ± 1.55	a	142	larval mass

[1]Means within a grouping followed by the same letter are not significantly ($P > 0.05$) different; values represent non-transformed data; statistical analysis was performed on transformed data.
[2]Total ticks refers to the total number of females, males, and nymphs.
[3]Larval mass refers to larval ticks counted as masses rather than individuals.

Table 3. Influence of ivermectin treatment on densities of lone star tick life stages in 1995.

Treatment	Mean/Sampling Day ± SD[1]		n	Stage
ivermectin-treated	2.07 ± 3.29	a	95	female
non-treated	1.10 ± 2.14	b	96	female
ivermectin-treated	1.72 ± 2.35	a	95	nymph
non-treated	1.41 ± 6.66	b	96	nymph
ivermectin-treated	5.23 ± 5.07	a	95	total ticks[2]
non-treated	4.44 ± 7.38	b	96	total ticks

[1]Means within a grouping followed by the same letter are not significantly ($P > 0.05$) different; values represent non-transformed data; statistical analysis was performed on transformed data.
[2]Total ticks refers to the total number of females, males, and nymphs.

Treated Area. Adult female tick abundance in the treated area was significantly different between years. Significantly more females were collected in 1995 than 1994 (Table 4). No significant difference existed between the number of nymphs collected in 1994 and 1995 (Table 4). Total ticks collected in the treated area were significantly different between years, with more collected in 1995 than 1994 (Table 4).

Non-treated Area. Numbers of adult females collected in the non-treated area also were significantly greater in 1995 than in 1994 (Table 5). The number of nymphs collected were significantly different between years, as fewer nymphs were collected in 1995 than 1994 (Table 5). Total ticks collected were not significantly different between 1994 and 1995, indicating no significant change from year to year (Table 5).

Table 4. Influence of year on densities of lone star tick life stages in the treated area.

Year	Mean/Sampling Day ± SD[1]		n	Stage
1995	2.07 ± 3.29	a	95	female
1994	1.62 ± 4.1	b	144	female
1995	1.72 ± 2.35	a	95	nymph
1994	4.21 ± 7.23	a	144	nymph
1995	5.23 ± 5.07	a	95	total ticks[2]
1994	5.00 ± 2.78	b	144	total ticks

[1]Means within a grouping followed by the same letter are not significantly ($P > 0.05$) different; values represent non-transformed data; statistical analysis was performed on transformed data.
[2]Total ticks refers to the total number of females, males, and nymphs.

Table 5. Influence of year on densities of lone star tick life stages in the non-treated area.

Year	Mean/Sampling Day ± SD[1]		n	Stage
1995	1.10 ± 2.14	a	96	female
1994	0.41 ± 1.00	b	142	female
1995	2.40 ± 6.66	b	96	nymph
1994	9.40 ± 65.41	a	142	nymph
1995	10.06 ± 35.10	a	96	total ticks[2]
1994	10.01 ± 35.41	a	142	total ticks

[1]Means within a grouping followed by the same letter are not significantly ($P > 0.05$) different; values represent non-transformed data; statistical analysis was performed on transformed data.
[2]Total ticks refers to the total number of females, males, and nymphs.

Impact of ivermectin treatment is best demonstrated in the reduction of larval masses in the treated area. Based on significantly greater numbers of females in the treated area, one would also expect significantly greater numbers of larval masses; however, no significant differences were found between treated and non-treated areas, suggesting that ivermectin treatment reduced female tick reproductivity. Significant differences in the numbers of total adults and nymphs in the treated and non-treated areas can perhaps be explained by complex two year life cycle of the lone star tick. Decreases in the densities of lone star ticks in the treated area from 1994 to 1995 are apparent, though not statistically different. Numbers of adults in both areas increased, indicating favorable environmental conditions for tick survival. Numbers of adults and nymphs in the treated area might have been much greater without the reducing effect of ivermectin treatment. Large collections of nymphs collected by dragging at a single site in the non-treated area on two consecutive sampling days may also explain some of differences in the treated and non-treated areas. Nymphal numbers >300 were collected from a single drag. These large collections would have influenced statistical analysis.

A study continued for more than two years may further define the impact of ivermectin on lone star ticks. A longer study also would allow for comparison of 1994 and

1995 larval masses, revealing the impact of ivermectin on female tick reproduction.

Seasonality of Lone Star Ticks in eastern Tennessee

High populations of lone star ticks were observed in the treated and non-treated areas of Fairfield Glade during 1994 and 1995. Of all the ticks collected, greater than 99% were lone star ticks. Five *D. variabilis* adults were collected during this study with flannel tick drags.

Seasonality of the lone star tick was determined by closely evaluating collection data from the non-treated area in 1994 and 1995. Mean numbers collected in 1994 and 1995 using flannel drags were 1.08 larval masses, 10.6 nymphs, and 1.20 adults per 600m of drag collection and 1.99 nymphs and 1.51 adults per 6-hour carbon dioxide sample.

Sampling date for collected larval masses, nymphs and adults was significantly different throughout the study season regardless of sampling method. Each of the three life stages exhibited definite patterns of occurrence.

1994. Larval masses were collected between 21 July and 19 September but were most abundant in late August (Fig. 7). Nymphs were collected from 18 April through 7 September; however seasonal distribution varied between sampling methods (Fig. 8). Nymphs collected by dragging increased from 29 April to 12 May, dropped during June and early July, and then increased slightly until 2 August. Numbers of

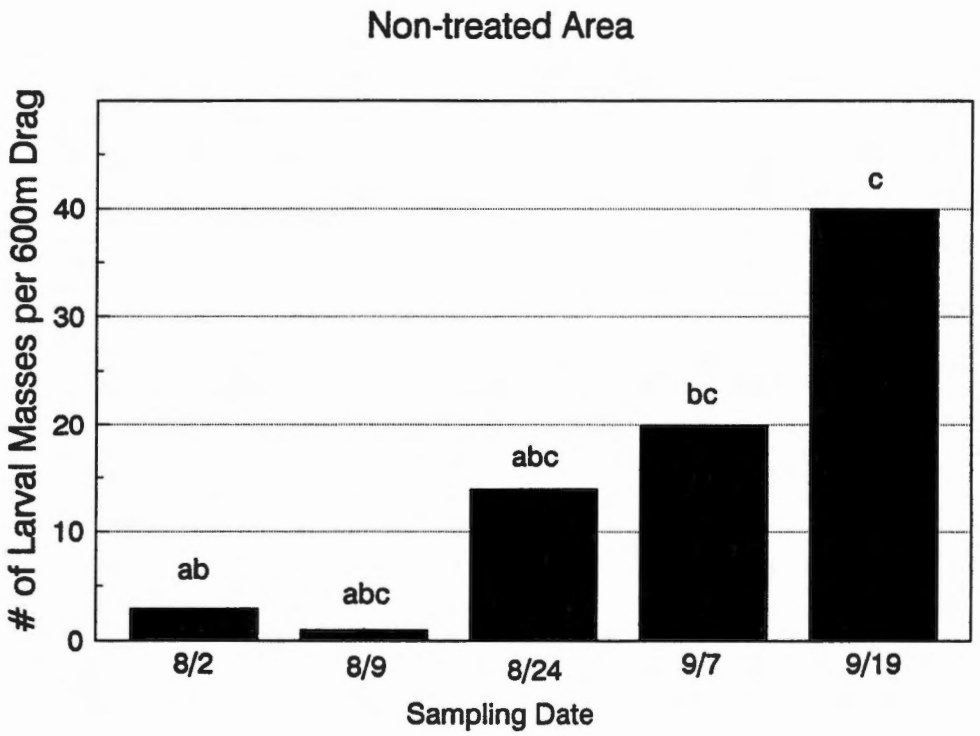


Fig.7. Seasonal abundance of larval masses collected by drag sampling in the non-treated area for 1994. Sampling dates with the same letter above are not significantly different.

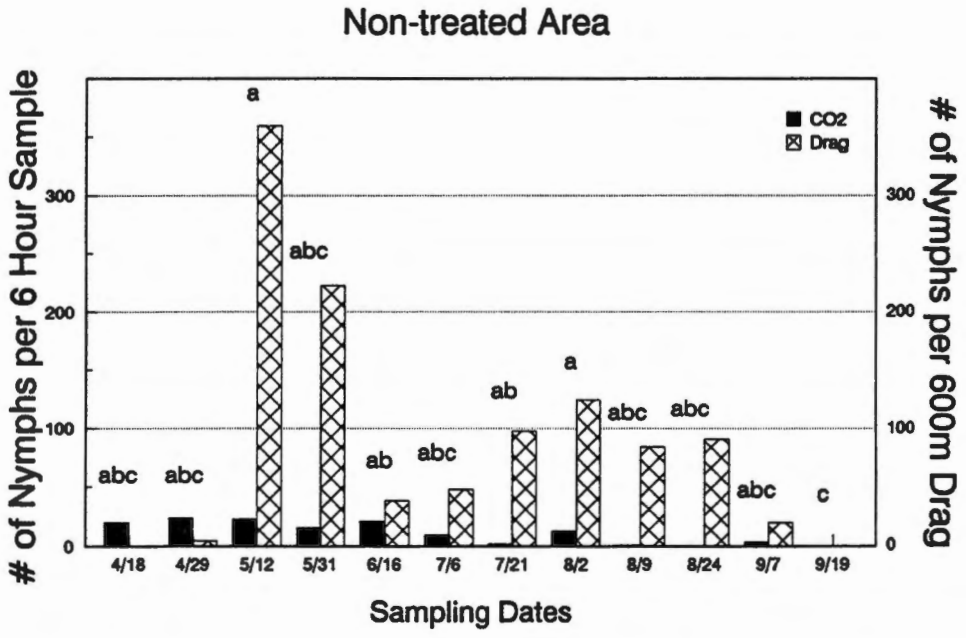


Fig. 8. Seasonal abundance of nymphs collected in the non-treated area for 1994 using carbon dioxide and drag sampling. Sampling dates with the same letter above are not significantly different.

nymphs then decreased and none were collected after 7 September. Carbon dioxide collections of nymphs remained steady from 18 April until 2 August. Numbers of adults also varied with sampling method (Fig. 9). Adults were collected by flannel drags from 18 April to 9 August with peaks in May and June. Numbers of adults dropped to zero after 9 August, whereas nymphs were collected until 7 September (Fig. 8). Adults were collected with carbon dioxide from 18 April until 6 July. No adults were collected with carbon dioxide after 6 July.

1995. Seasonality of nymphs again varied by sampling method (Fig. 10). Nymphs were collected from 31 March until 3 July. Nymphs were collected by dragging from 10 April until 3 July with densities peaking on 8 and 21 June. Nymphs were collected with carbon dioxide from 31 March until 3 July with densities peaking on 28 April. Numbers of adults also varied between sampling types in (Fig. 11) and were collected from 31 March until 3 July. Drag-collected adults were recovered on all sampling dates and numbers of adults peaked on 8 June. Adults collected with carbon dioxide also were found on all sampling dates. Peak adult densities occurred on 31 March.

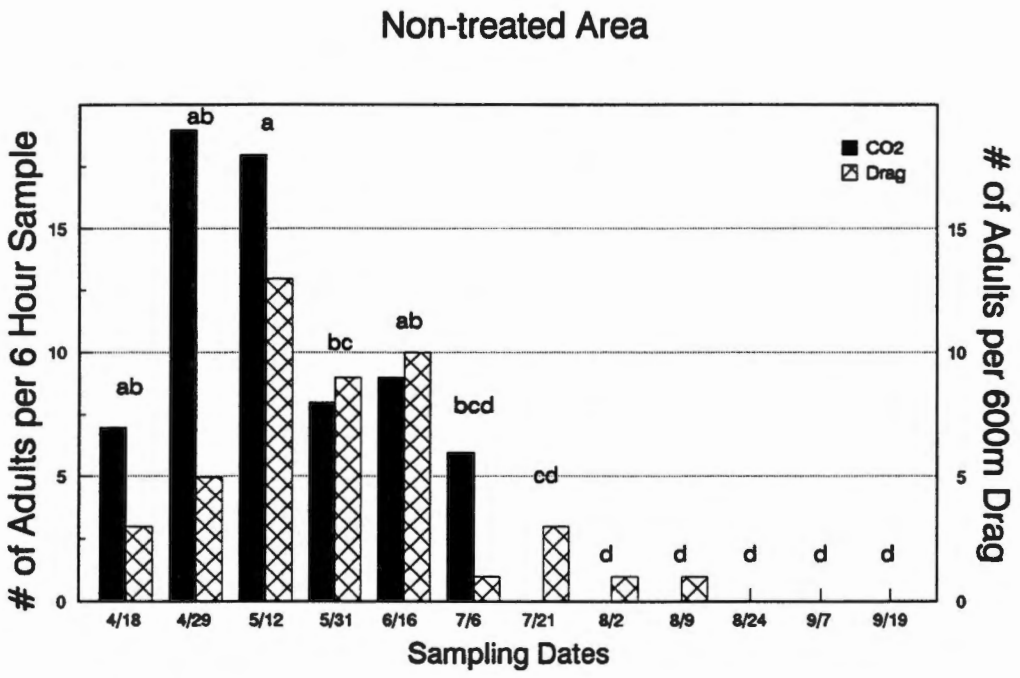


Fig 9. Seasonal abundance of adults collected in the non-treated area for 1994 using carbon dioxide and drag sampling. Sampling dates with the same letter above are not significantly different.

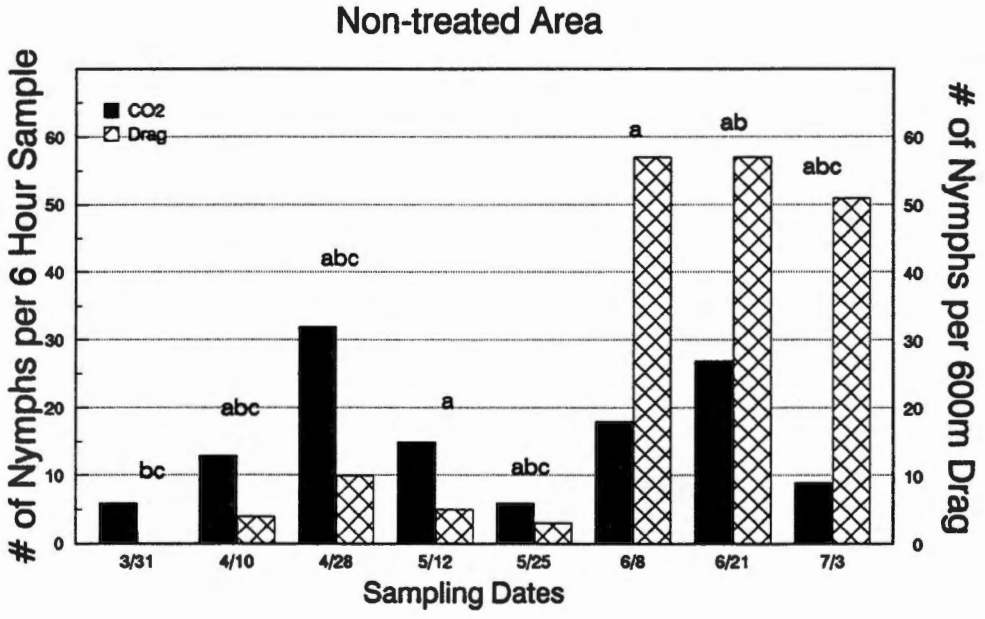


Fig.10. Seasonal abundance of nymphs collected in the non-treated area for 1995 using carbon dioxide and drag sampling. Sampling dates with the same letter above are not significantly different.

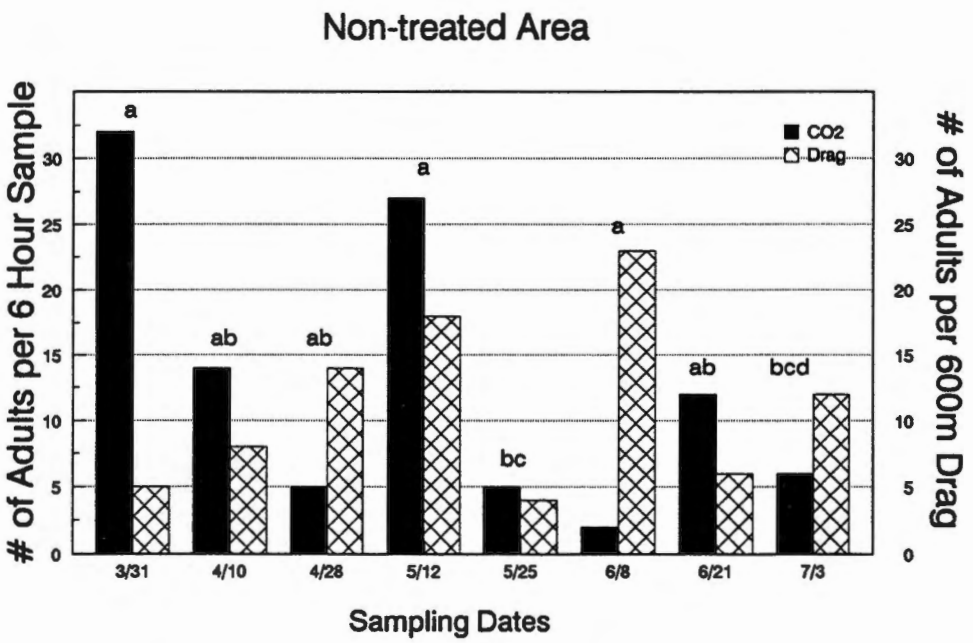


Fig.11. Seasonal abundance of adults collected in the non-treated area for 1995 using carbon dioxide and drag sampling. Sampling dates with the same letter above are not significantly different.

Seasonal trends for adults and nymphs were consistent in 1994 and 1995 and population peaks followed similar monthly trends. Extension of the study season through September 1995 would likely reveal similar drops in adult collections to zero in August and nymphs in early September. Seasonal abundance of larval masses in 1995 could also be determined. Patterns of seasonal abundance of each of the three lone star tick life stages were fairly consistent with those previously found in central Georgia (Davidson et al. 1994). Differences in abundance due to sampling methods also have been reported previously (Hair and Howell 1970, Kinzer et al. 1990). Pattern variations are due to tick behavior in response to temperature differences, which affect the vulnerability of ticks to sampling methods. During the periods of cooler temperatures in early spring, adults and nymphs are found primarily on the forest floor, making them more susceptible to carbon dioxide sampling. As temperatures increase during late spring and summer, ticks ascend vegetation and are more likely to be collected by dragging.

Comparison of Lone Star Tick Collections form Wooded Areas and Mowed Roadsides

Densities of developmental stages of the lone star tick were significantly different between sampling locations within a sampling site (Table 6). Densities of females in

Table 6. Influence of sampling location on densities of lone star tick life stages.

Loc. Type	Mean/Sampling Day ± SD[1]	n	Stage
woods	1.24 ± 2.72 a	143	female
grass	1.14 ± 2.83 a	143	female
woods	9.07 ± 31.26 a	143	nymph
grass	2.586 ± 4.74 b	143	nymph
woods	1.69 ± 3.91 a	143	total ticks
grass	1.51 ± 3.40 b	143	total ticks
woods	0.304 ± 1.595 a	143	larval masses
grass	0.232 ± 0.91 a	143	larval masses

[1]Means within a grouping followed by the same letter are not significantly ($P > 0.05$) different; values represent non-transformed data; statistical analysis was performed on transformed data.
[2]Total ticks refers to the total number of females, males, and nymphs.
[3]Larval mass refers to larval ticks counted as masses rather than individuals.

wooded areas and grassy roadsides were not significantly different. Significantly greater numbers of nymphs and total ticks were collected from wooded areas than from grassy areas. Larval masses, like adults, were not significantly different between locations.

CHAPTER IV

CONCLUSIONS

Statistical analysis of numbers of lone star ticks collected in 1994 and 1995 indicated no significant effects of ivermectin treatment on tick densities. However, densities of ticks in the treated area were reduced from 1994 to 1995, though they did not differ significantly. The difference from 1994 to 1995 indicates a slow decrease, possibly due to ivermectin treatment. Although more ticks were collected in the treated area for the 1994 and 1995 study seasons, reduction of larval mass densities in the treated area to four times below the expected number suggests that ivermectin affects densities of lone star ticks. The treated area had significantly more females in 1994 which should have produced significantly more larval masses. However, numbers of larval masses were not significantly different between treated and non-treated areas. Due to the complex two-year life cycle of the lone star tick, an extended study with more treatment and sampling seasons is needed to gain an understanding of the impact of ivermectin on populations of lone star ticks.

Seasonality of ticks collected in the non-treated area in 1994 and 1995 closely followed previous seasonality research in central Georgia by Davidson and co-workers (1994). Lone star tick population cycling and collection

peaks in the non-treated area approximated those found in central Georgia, as did differences in seasonality due to sampling type.

Comparisons of samples made in wooded and grassy areas revealed that significantly greater numbers of nymphs were collected in the wooded areas. No significant differences were found between wooded and grassy areas for numbers of females and larval masses.

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