ECOLOGY OF THE FREE-LIVING STAGES OF THE LONE STAR TICK, *AMBLYOMMA AMERICANUM* (LINNEAUS)

By

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ECOLOGY OF THE FREE-LIVING STAGES OF THE LONE
STAR TICK, AMBLYOMMA AMERICANUM (LINNEAUS)

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PREFACE

This thesis is a summation of a habitat distribution study on free-living Amblyomma americanum (Linneaus) in part of Cherokee county, Oklahoma. A plant preference study was also conducted in the laboratory and in the field.

Certain habitats and plant species were found to be more desirable for ticks than others. The ticks were found more frequently in humid, shaded areas than in open sunny areas.

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CHAPTER I

INTRODUCTION

The lone star tick, *Amblyomma americanum* (Linnaeus), is one of the most important pests of man, livestock, and wildlife in the southeastern United States. Death to wildlife, weight losses in livestock, and annoyance to man via irritating bites can be attributed to it. More important, some diseases of man are transmitted by this tick species, including Rocky Mountain spotted fever and tularemia. Its notorious reputation as a pest of man probably keeps many tourists away from beautiful areas in the southeastern United States, eastern Texas, and southern and eastern Oklahoma. Otherwise, this area has a vast potential as a recreational paradise.

Three-host ticks, such as *A. americanum*, spend much of their lives on plants awaiting a host. Once the female has obtained a blood meal from her host, she seeks shelter in a shady humid place, often in thicker vegetation or under objects near an animal trail. There she lays her eggs which will hatch in three to six weeks. The larvae usually climb to or near the top of a plant to await a host. When a potential host touches the plant or the ticks, the ticks cling to the animal and crawl to a favorable site for attachment. The nymphal and adult ticks follow similar life cycle patterns. While plants are not required to complete the life cycle, they enable the tick to survive longer and to find a host more readily.

The purpose of this study was to determine the type of habitat A. americanum occurred in most frequently, to study larval tick behavior on plants, to determine if a plant species preference exists, and to determine where and in what stages the ticks overwinter.

Habitat studies were concerned with the distribution of the lone star tick over 10 study areas in Cookson Hills State Game Refuge. Factors studied included: vegetation type, height of the vegetation, density of the vegetation on the floor of the woods, density of the leaf litter on the forest floor, and the prevalence of ticks at various distances from the woods' edge into the prairie. The relationship of the tick populations to the common trees and ground plant species in the area was also considered.

Little information is available on where and how A. americanum overwinters. The overwintering study was conducted to determine the specific habitat in the decomposing leaf litter, or in the soil below the leaves.

The larval movement study was made to determine the behavior of ticks on plants to various external stimuli. The plant preference study was conducted to determine whether certain species of plants attracted or repelled A. americanum. It has been noted that certain plants in other parts of the world acted as repellents to ticks.

Biological data from these studies could assist scientists in developing control procedures for this pest. For instance, Clymer (1969) found that clearing of woody vegetation yielded 40-50% control of all life stages of A. americanum. Such findings with supplemental biological information could simplify the control of the tick and eventually reduce the tick population to a level where it does less harm.

CHAPTER II

REVIEW OF THE LITERATURE

Many early workers observed ticks on vegetation with their fore-legs spread awaiting a host. Aristotle (384-322 B.C.) believed that ticks came out of the grass. Herms and James (1961) indicated that when ixodine (hard) ticks reached maturity on the host they fell from the animal and laid eggs. After the larvae emerged and climbed up low shrubbery, weeds, or grass, they were then ready to cling to any suitable passing host. Enormous numbers can often be encountered along animal trails. In some areas, large numbers of nymphs and adults will climb up the branches and twigs of low shrubbery, where they are in excellent position to attach themselves to larger animals that brush against the vegetation.

Eddy (1940) found that *A. americanum* was distributed throughout much of southern and eastern Oklahoma. Bishopp and Trembley (1945) stated that the lone star tick was found in large numbers in wooded areas, particularly where underbrush was dense, such as along river bottoms, in prairie areas, and in canebreaks. Calhoun and Alford (1955), while studying tularemia in lone star ticks in Arkansas, noted that on a 160 acre farm the highest concentration was found in a small wooded area measuring approximately 75 $yds.^2$. Concentration of cattle within this small area for shade as well as the high humid conditions along a wet weather creek bed, afforded excellent habitat for the large population of lone star ticks found there. Lancaster (1957) found that brushy pastures were more heavily infested with A. americanum than open, improved pastures. The typical Arkansas tick habitat is brushy or timbered areas, which are generally unsuited for cultivation. Lancaster (1957) found that 69.6% of the lone star ticks taken in his survey were from brush, 18.4% from tall broomsedge (Andropegon virginicus L.) along a cattle trail, 5.1% from wooded underbrush, 4.4% from open woods, 1.5% from open pasture, and 1% from sedge. Sonenshine, et al. (1966) showed that A. americanum was distributed more or less uniformly in four woody vegetative types in Virginia. However, the combined grasses-and-herbs vegetative type had markedly lower adult and nymphal densities when compared to the woody type.

Arthur (1948) found that Ixodes ricinus (Linneaus), in South Wales, favored rush (grass-like) over bracken (fern). In central Wales bracken was infested 3.4 times more than rush, while in northern Wales bracken was infested 1.4 times more often than rush. He found that the pH of the tick infested areas ranged from 4.4 to 5.2. One of his suggested control procedures was liming, to raise the pH.

Probably, several factors cause the patchy distribution of ticks. Milne (1943), in northern England, suggested that since I. ricinus larvae from one egg mass are concentrated within a small diameter, probably a few inches, hundreds may be picked up at one time by one sheep; hence, they will drop off engorged at about the same time. Nymphs, therefore, should also tend to occur in patches, though these will be relatively large as compared to larval forms. Milne (1946) in northern England studies found that I. ricinus populations were lower in sheep pens due to vegetative cover. Vegetation was too thin and the ground was too dry to support ticks. Milne (1944) reported that surface geology, which affects vegetative cover, also affected tick distribution. He found a positive correlation between the mat thickness and the sheep-tick population density. The thicker the mat, the higher the tick population regardless of the vegetative type.

MacLeod (1936) stated that the problem of the causes underlying the local distribution of ticks is simplified by the habit of feeding they have adopted. Being dependent on the will of their host for dispersal, ticks can exercise no selection in relation to their habitat and habitat conditions, he said. He continued, thus the factors which will determine the suitability of a habitat are primarily edaphic: the nature of the soil, presence or absence of a humus-forming layer, and vegetative type.

El' manov (1930), Nakanura and Yajima (1942), and Sudachenkov (1941), working with I. ricinus, Haemaphysalis bispinosa Neumann, I. persulcatus Schulze., as well as other ticks, found that these pests were most abundant in areas where there was a mixture of prairie and woods. They noticed few ticks in open prairies with no trees. They reported that these ticks were most abundant in low moist areas. All ticks, however, were not confined to high humidity and regions of mixed vegetative types, but apparently A. americanum was.

The availability of a suitable host and relative humidity are two major factors limiting the distribution of many ticks. Lancaster and Macmillan (1955), in Arkansas, demonstrated that a lack of moisture often limited survival of the lone star tick. Eggs kept at 69%

humidity by this worker would not hatch. He also noted that humidity in brush ranged slightly higher than in the open pasture during the day. More significantly, he noted, the humidity in the brush stayed about 50% for a longer time than it did in the open pasture. Sonenshine and Tigner (1969) reported that no A. americanum eggs hatched at 55% relative humidity, 6.4% hatched at 60% R.H., 15.4% hatched at 65% R.H., 77.5% hatched at 85% R.H., and 95.4% hatched at 95% relative humidity.

Central Oklahoma studies by Bruner (1931) showed that the relative humidity of a woodland was significantly higher than the relative humidity of a prairie. He noted that on moist, cloudy days the humidity of the communities were about the same. On hot, dry days the humidity of the prairie was as much as 26% lower than in the woodland. In 1925, the humidity of the prairie averaged 40%, while the relative humidity of the woodland averaged 58%.

In Scotland, I. ricinus survival was determined by microclimate and humidity, but activity was determined by air temperature (MacLeod 1938). Humidity was found to have no effect on activity beyond the indirect one of affecting duration of life.

Some species of ticks can move great distances, considering their size and means of locomotion. Smittle, et al. (1967) found that marked A. americanum adults migrated up to 75 ft. in 72 hrs. and 95 ft. in 11-12 weeks. Such migration was predominantly towards and into shaded areas. Nymphs were observed to move up to 55 ft. Sonenshine, et al. (1966) reported that marked Dermacentor variabilis (Say) moved up to 182 ft. and Marikovskii (1945), in Russia, found that I.

persulcatus moved 3-12 m per hr., and D. silvarum Olenev. moved 12-36 m per hr.

Smith, et al. (1946) reported that ticks were attracted to areas or objects that contain the scent of man or a natural host. He stated that the most important factor in the patchy distribution of ticks is probably the habits of the host. In a tick-host survey by Clymer (1969) in eastern Oklahoma, the leading hosts of A. americanum were white-tailed deer and cattle.

MacLeod (1938) reported that Scottish deer lie resting in mild weather for most of the forenoon and afternoon. They occupied the same favorite resting places day after day and sometimes year after year. He felt that it follows that engorged ticks will tend to be concentrated in these lairs, and thus would be able to immediately find a host when they had molted. Therefore, he stated, the tick population in a deer forest is concentrated on the deer. He noted that, by far, the greater number of ticks attached to deer are picked up in their lairs, and most of them are dropped there when they are engorged.

Somov and Shestakov (1963), while studying spotted deer in Russia, found that quantitative infestations of deer with H. japonica var. douglasi Nuttall and Warburton differed considerably from infestations on cows. They noted that in May and June, when an average of 30-50 ticks were found on cows, several thousands were found on deer.

Michael (1965), while studying the white-tailed deer, Odocoileus virginianus Zimmermann, observed that most marked deer appeared to have several favorite areas in which they bedded. If these sites were not near where they were feeding, the deer bedded in any suitable cover nearby.

McMahan (1966) found that the white-tailed deer in Kerr wildlife management area, Texas, were generally observed to feed in particular sites. Such sites appeared to correlate with proximity to overstory cover. Livestock, however, were less discriminating in their foraging habits. A preferred deer feeding site in Kerr refuge was one that contained palatable forbs. McMahan also found that free ranging deer, whenever possible, avoided pastures stocked with livestock and instead made use of the land area being rested from livestock grazing.

According to Bartlett (1938), woods' edges and good deer habitat are synonymous. He stated that forest lands in which the crowns of trees produce virtually a closed canopy, and shade the ground so that an abundance of low-growing shrubs and bushes cannot grow, make poor deer habitat. Deer are found most abundantly along the margins where the heaviest desirable cover and the greatest amount of available food in the form of low-growing shrubs and bushes are found.

The foods of the white-tailed deer also affect tick distribution. Among the favorite deer foods in Missouri according to Dalke (1941) are: grape, greenbrier, red maple, hard and silver maple, most oak species, black oak acorns, pine, sassafras, and winged sumac. Species showing lower use were: blackberry, American elm, slippery elm, winged elm, hickory, lespedeza, persimmon, plum, poison ivy, sedges, rushes, and black walnut.

Review of Tick Movement Studies

A sound understanding of tick behavior on vegetation may lead to ways to control them. Several studies have been made on tick movements. MacLeod (1939), in Scotland, found that within a certain temperature range, *I. ricinus* are negatively geotropic, while above and below this range they react positively. He suggested that in nature, ticks climb the vegetation or descend to the roots depending on the ambient temperature. Ticks, which are at the roots of the vegetation in the winter, are at this season exposed to the inactivating influence of low temperatures and will not normally be capable of attaching to browsing animals. This worker felt, however, that ticks inactivated by cold responded immediately to a rise in temperature. Ticks then resumed activity so occasionally they could be found on animals during the winter.

Wilkinson (1953) stated that in *Boophilus microplus* (Canestrini), the main stimulus governing ascent of the grass blades, appeared to be positive phototaxis to moderate light intensities, rather than negatively geotaxis. Larvae tended to stay sheltered from direct sunlight. Larvae in the field were found to be more exposed in the early morning, often at the tops of grass stalks. Lees and Milne (1951) noted that all stages of *I. ricinus* were found on the upper one-third of the vegetation (grass) more frequently than the lower two-thirds.

Lees (1948) stated that an important physical feature of rough moorland grazings, which formed the main habitat for *I. ricinus*, was the steep humidity gradient within the vegetation layer. The tendency to remain near the tips was assisted at first by the high humidity response, for ticks walking on the stem lattice avoided the high humidity near the roots. After an unsuccessful period of waiting, the ticks come to rest at the roots, take up water from the damp atmosphere, and then are prepared for a future period of activity at the tips, he stated. Other authors, however, have found that many species of ticks do not take up atmospheric moisture.

Lees and Milne (1951) found that I. ricinus tend to avoid the direct rays of the sun. Positions shaded from the sun were selected by 128 out of 257 I. ricinus under observation. They found that I. ricinus was also affected by the wind.

Plant Preference Studies

There has been very little work done on plant preferences of ticks. Marikovskii (1945) noted that quite often ticks were found at the end of the fine twigs of dichromatic lespedeza (Lespedeza bicolor). In addition to this, he noted that a preference was given to twigs that overhung a free area at the periphery of groups of shrubs near footpaths in the woods. Menendez (1924), working in Puerto Rico, found that the grass Melenitis minuteflora did not destroy the tick B. annulatus (Say), but repelled it. The movement studies by Smittle, et al. (1967), Sonenshine, et al. (1966), Smith, et al. (1946), and Marikovskii (1945) indicate that adult and nymphal ticks can move far enough to select a plant if they prefer it.

CHAPTER III

MATERIALS AND METHODS

Study Area - Location

Cookson Hills Refuge, located in southeastern Cherokee and southwestern Adair counties, Oklahoma, was selected as the study area because of high tick populations and the availability of land for study. This area has a high deer population which has been affected severely by A. americanum, the lone star tick.

Ten study areas were set up in the southwestern corner of the refuge. These areas were as follows:

Study Area No. 1 (S22 R23E T14N) was a 40 acre prairie of Johnsongrass [Sorghum halepense (L.) Pers.], broomsedge (A. virginicus L.), and Korean lespedeza [L. striata (Thunb.) H & A], plus 20 acres of oak-hickory woods fringed with winged elm (Ulmus alata Michx.) and sassafras [Sassafras alibidum (Nutt.) Nees].

Study Area No. 2 (SE¼ SW¼ S15 R23E T14N) contained 4 acres of broomsedge prairie and 8 acres of oak-hickory woods.

Study Area No. 3 (SE¼ SE¼ S15 R23E T14N) consisted of 14 acres: 3 acres of broomsedge and Johnsongrass, 5 acres of sassafras-persimmon brush, 1½ acres of broomsedge prairie, and 4½ acres of oak-hickory woods.

Study Area No. 4 (SW¼ NW¼ S23 R22E T14N) consisted of 6 acres: 3 of Johnsongrass-broomsedge prairie and 3 of oak-hickory woods.

Study Area No. 5 (NE¼ SE¼ S15 R23E T14N) consisted of 1½ acres of Johnsongrass, 1½ acres of persimmon-sassafras woods and 3 acres of oak-hickory woods.

Study Area No. 6 (NW¼ SE¼ S15 R23E T14N) consisted of 2½ acres of prairie and 3½ acres of oak-hickory woods.

Study Area No. 7 (NW¼ SE¼ S15 R23E T14N) was a flood plain consisting of 12 acres of oak-hickory forest.

Study Area 8 (SE¼ NE¼ S15 R23E T14N) consisted on ½ acre of serica lespedeza and 4½ acres of oak-hickory woods.

Study Area 9 (NE¼ NE¼ S11 R23E T14N) was a 3 acre pine woods.

Study Area 10 (NW¼ NE¼ S12 R23E T14N) was located on a hillside near a spring-fed stream. It contained 1 acre of herbaceous prairie and 4 acres of oak-hickory forests.

Sampling Procedures for *Amblyomma americanum*

Vegetative Preference Studies

Within each study area, drag strips were marked off at 25 yd. intervals so that a drag strip had another strip 25 yds. on each side and 25 yds. from each end. While or after the strips were marked, samples of the active *A. americanum* were taken by the drag and sweep method described by Clymer (1969).

A standard sweep net, 15 in. in diameter, was used along with a drag (3 x 6 ft. piece of muslin attached to a 3 ft. dowell pin) to estimate the population. The sweep net was swung once for each step taken while the drag was pulled over the vegetation for 25 paces. After the sample was taken, the number of ticks on each side of the drag and sweep were counted according to stage and sex and recorded.

This gave an estimate of the tick population in the 25 $yd.^2$ area covered by the drag.

All samples were taken on warm fair days between 8:00 a.m. and 7:00 p.m. Samples were taken as quickly as possible to get similar environmental conditions within a given area. Samples of each area were taken between June 15, 1969 and July 16, 1969. Drag strips in sample areas 1, 3, 6, and 7 were sampled twice, while all others were sampled once.

After sampling was finished, the vegetation of each drag strip was studied. A list of the common trees and herbs was recorded along with density of the trees and herbs, the height of the trees and herbs, and the amount of leaf litter found in the sampled area.

The most common plant species in each area was determined by visual observations while walking from one end of the strip to the other. In the woods, the visually dominant tree species was recorded along with the visually dominant floor plant species. The number of samples taken and the number of adult and nymph A. americanum per sample were figured for each dominant tree habitat type.

Effect of Habitat Type on Tick Populations

This study was conducted to determine the plant communities that support higher tick populations. The dominant tree habitat types divided into three groups (Figure 1): (1) persimmon-sassafras, a low brushy woods that grows between the taller trees and the prairies, (2) post oak-blackjack, found on dry hills, and (3) oak-hickory, found in locations with more moisture. Prairie habitat types were divided

Figure 1. Four Vegetation Types Found in Cookson Hills State Game Refuge. A, Upland Oak-Hickory Forest; B, Persimmon-Sassafras; C, Bottomland Oak-Hickory Forest; D, Prairie.

into three groups, depending on their relationship to the woods. Groups within prairies were: (1) open prairie (2) edge of prairie next to woods, and (3) small openings in woods.

The main tree species in the persimmon-sassafras group were persimmon (Diospyros virginiana L.) and sassafras (S. albidum). Other trees in this group were winged sumac (Rhus copallina L.), smooth sumac (R. glabra L.), winged elm (U. alata), and occasionally black locust (Robinia pseudo-acacia L.).

The dominant trees in the post oak-blackjack oak woods were post oak (Quercus stellata Wang.), and blackjack oak (Q. marilandica Muenchh.). Other trees found in this community were black hickory (Carya texana) and Celtis sp.

The oak-hickory group contained many species of trees. The main trees included black oak (Q. velutina Lam.), red oak (Q. rubra L.), several hickories (Carya spp.), and several elms (Ulmus spp.).

Influence of Vegetative Density on Tick Numbers

This study was conducted to determine the effect of woodlot floor vegetation density on A. americanum populations. Density of vegetative growth was determined by visual observation while walking the drag area. Less than 25% coverage of the ground by undergrowth was classified as light; 25-75% coverage, medium; and over 75% coverage, thick. On this basis, scattered undergrowth was thin, moderate undergrowth was medium and dense undergrowth with no ground visible was thick. Tick samples for each density classification were totaled and the average numbers of male, female and nymphal A. americanum per sample were calculated.

Influence of Leaf Litter on Tick Abundance

This study was made to determine the effect of leaf litter density on tick populations. Density of the leaf litter was estimated in a manner similar to the estimation of vegetation density. Classifications of leaf litter density were: light, less than 25% coverage; medium, 25-75% leaf litter coverage; and thick, over 75% litter coverage. Samples taken from each classification were totaled and an average was determined for male, female, and nymphal A. americanum in each classification.

Effect of Shading by Woodlots on Tick Numbers in the Ecotone

This study was conducted to determine the effect of shading on tick populations. Samples taken along the edges of woodlots were divided by the four cardinal directions from the woodlot: north, south, east, and west. Samples from each direction were totaled and the average numbers of male, female, and nymphal A. americanum were determined.

Effect of Height and Type of Vegetation on Tick Numbers

This study was done to determine the effect of vegetation size on tick populations. Grasses with herbs were divided into three categories according to height: low, less than 2 ft. tall; medium, 2-4 ft. tall; and tall, more than four ft. tall. Trees and shrubs were divided into four categories: conifers any height; deciduous low, less than 20 ft. tall; deciduous medium, 20-40 ft. tall; and deciduous tall, over 40 ft. tall. The samples for each habitat type were totaled and the average numbers of each stage and sex of A. americanum were determined for each category.

Effect of Distance from the Ecotone on Tick Populations

This study was conducted to determine the effect of increasing distance from the ecotone into the prairie on tick populations.

Samples were taken along the ecotone at the north edge of a wood-lot. Samples were taken at 0, 25, 50, 75, 100, and 125 yard intervals into a prairie. Amblyomma americanum taken from each interval were grouped according to stage and sex and were totaled.

Aggregation Study

To find how spotty A. americanum distribution was in the study areas, the highest one, five and ten percent of the total tick samples were determined. From this the percentage of the total ticks in the most heavily infested areas was determined. One thousand drag samples were taken during this study so the ten samples with the highest number of ticks were combined. The total of the ten highest samples was divided by the total of the 1000 drag samples. This gave the percentage of the total number of ticks in one percent of the area.

Population Estimate of Study Area No. 1

An estimate of the active A. americanum population in Study Area No. 1 was made to determine the number of active ticks at various times of the year and to indicate the severity of the problem there.

Two estimates of the active A. americanum population in the study area were made during 1969. The first estimate was made June 15, 16, and 17. The second estimate was made July 13, 14, and 15. Samples were taken systematically over the 60 acre study area. The population estimates were figured on the average number of ticks per acre. The estimates were then fitted to a graph of the seasonal distribution of A. americanum. The seasonal distribution was determined by taking four random samples in each of three plots in the same general vicinity at weekly intervals.

Larval Behavior Studies

This study was conducted to determine the effect of sunlight on larval behavior.

Masses of A. americanum larvae were located on plants by walking along game and livestock trails and checking the vegetation along the edge of the trails. When a mass of larvae was located, the area was marked with forester's tape. Cylinders, 3 ft. high and 12 ft. in circumference, made of woven wire were placed around the masses of larval ticks to keep deer, cattle, and other animals from disturbing them during the ensuing study periods.

Twelve masses of larvae were located during the first part of August, 1968 and six others were located during the first of September. All but one of these masses of larvae were found in the shade. One mass was moved by cutting off the plant at the base and putting it into the ground at another site.

Each observation included the ambient temperature, the relative humidity, the percentage of shade in the cylinders, the time of the day, the direction of the sun, the weather conditions, and the location of the ticks on the plants. The air temperature and humidity were measured with a Friez aspirator type psychrometer at the level where most of the larvae were localized on the plant. The percentage of shade was estimated by observing the amount of shade in the wire cylinder. The general location of the mass of ticks on the plant was related to the direction of the sun.

Height of A. americanum on Vegetation

A study was conducted to determine the range in height of the stages of A. americanum on vegetation. A yard-stick was used to measure the height of adults, nymphs, and larvae on several different plant species.

Plant Preference Studies in the Field

Preliminary observations were made to determine the plant "host" range of the lone star tick during 1968 in Study Area Nos. 3 and 10 and a highly infested area in Cherokee State Game Refuge. The tick populations in all these areas were high. Each observation was made by selecting equal numbers of plants in the area. Each observation consisted of the kind of plant (family, genus, or species), the height of the tick on the plant, and the number of each stage of A. americanum on the plant. A list of the plants that lone star ticks were found on during this study was made. The height preference of each stage of A. americanum was determined.

Laboratory Plant Preference Studies

Six plant species were selected because of availability and the abundance of ticks on the plants during field observations. The plants selected for laboratory study were: sericea lespedeza [L. cuneata (Dumont) G. Don], tick clover (Desmodium spp.), buckbrush (Symphorocarpos orbiculatus Moench), Johnsongrass (S. halepense), sedgegrass (A. virginicus), and saw greenbrier (Smilax Bona-nox L.).

A turntable was used for the plant preference study to equalize the relative humidity, temperature, and light on each plant during the tests. A 3/8 inch high disc of plywood 3 ft. in diameter was mounted in the center of a rotary bearing from a lawn mower. The base of the bearing was mounted on a one ft.^{2} platform 5 in. high. A fan belt from an electric motor mounted on the platform was connected to the rotary bearing and turned the table at one r.p.m.

A sheet of clear cellulose nitrate 6 in. wide was stapled and glued around the edge of the plywood base, thus, forming a bowl 5½ in. deep. White petroleum jelly was spread around the base and the top of the acetate on the inside. The acetate and the petroleum jelly kept the ticks confined to the turntable.

The testing laboratory was air conditioned and the temperature was kept between 70 and 80 degrees Farenheit. The relative humidity ranged between 55% and 80% during the tests.

Six hypothetical points, each located 9 in. from the edge of the turntable, were plotted equidistant about the circumference of the table. The points were numbered from one to six.

Plants to be tested were collected in the field by breaking off pieces of the plants and placing them in plastic bags immediately to preserve their natural chemical and physical structure.

Adult A. americanum, used as test organisms, were collected in the field. The ticks were collected from vegetation, a sweep net, a drag, and the collector's clothes. They were then confined in a jar with small bits of grassy plants to keep the humidity high.

Three species of plants were selected for each experiment. Terminals of the test plants 9 in. long were cut off. Leaves were removed from the bulkier plants and their stems were shortened to equalize the volume of the three species. Two plant parts of each species of plant to be tested were placed on the turntable opposite each other.

Each test consisted of six ten-minute observations. Each species included two observations at each of the three pairs of points. The combinations were: 1,2,3; 1,3,2; 3,1,2; etc. This means that in 1,2,3 the first species of plant is on points one and four, the second species of plant is on points two and five, and the third species of plant is on points three and six. The changes were made because there was a difference between the pairs of points and this gave each plant an equal chance of being selected by the ticks during the test.

The percentage of ticks attracted to each species of plant was calculated. Percentages for each plant species were averaged, giving the percentage of the possible for each of the six species in relation to all of the other plants.

Studies on Overwintering *A. americanum*

Areas with high tick populations during the summer of 1968 were selected for sampling the *A. americanum* overwintering in the soil.

A soil sampler was constructed so that it would take a top soil sample 6 x 6 x 1½ in. in size. A 6 x 6 x 2 in. piece of pine wood and a 24 x 3½ in. piece of heavy tin nailed around it formed a dish 1½ in. deep for the standard sampler. The edge of the tin was sharpened to allow easy penetration of the soil by the sampler.

A sample was taken by throwing the sampler at random over a "ticky area", or by placing the sampler at the base of plants known to have had larvae of *A. americanum* on them during August and September of 1968. After throwing the sampler, it was then turned in the appropriate direction, the sharp edge down, and pushed into the soil. The sampler was picked up and all the leaf litter was removed from the area marked by the sampler and placed into a labeled paper bag. The sampler was then placed on the bare area where the litter had been and pressed into the soil. A shovel was pushed under the sampler to prevent loss of soil. The soil in the sampler was then transferred to a paper bag which was properly labeled. All paper bags containing samples were stapled shut and put into plastic laundry bags. Twenty samples, 10 leaf litter and 10 top soil samples, were taken in each of two areas.

Berlese funnels were used in attempts to collect ticks from soil and litter samples. One-half of the leaf litter samples and one-half of the top soil samples were run for one week, then the other half of the samples was put into the funnel a week after sampling. The heat source was a 25 watt bulb in each funnel. These were used to allow the ticks to come out of the soil gradually. Ticks were collected in pint jars containing 50% ethanol. Each day the samples in the jars were poured through a 16 mesh per inch screen and examined for ticks. The ethanol was strained through a white cloth and returned to the jar.

CHAPTER IV

RESULTS AND DISCUSSION

Effect of Plant Communities on Tick Populations

Vegetation type had a significant effect on the tick populations found in the various study areas (Table I). The highest tick populations were found in brushy persimmon-sassafras groves and winged elm groves. Openings and trails through the woods had higher tick populations than the average wooded area. Post oak-blackjack oak communities averaged about twice as many ticks as the oak-hickory communities, while the prairie edge and the oak-hickory forest had about the same number of nymphs. The open prairie had about one-tenth as many nymphs and one-eighteenth as many adults as the prairie-woods ecotone. The main hosts of the lone star tick in the study area, according to Clymer (1969), were white-tailed deer and cattle. The principal habitat of the white-tailed deer is along the edge of the woods where there is plenty of browse and cover. This, and the higher humidity, as well as the lower temperatures along the woods-prairie ecotone and in the woods, probably accounted for the higher concentrations of ticks along the ecotone when compared to the prairie area.

The unprotected prairie provides plenty of sun exposure, which generally results in higher temperatures and lower humidity. According to Lancaster (1957), open prairie had a lower humidity than nearby brush during the mid-part of the day.

TABLE I

THE AVERAGE NUMBER OF TICKS PER SAMPLE[1/] FOUND IN DIFFERENT HABITATS IN COOKSON HILLS STATE GAME REFUGE, OKLAHOMA DURING JUNE AND JULY, 1969

Habitat of Community	No. of Samples	Males	Females	Adults	Nymphs
Open prairie	344	.1	.1	.2	.96
Forest prairie ecotone	177	1.2	1.6	2.8	8.5
Openings in woods	45	1.9	2.6	4.5	24.1
Woods:					
Persimmon-sassafras	83	2.0	2.0	4.0	68.4
Post oak-blackjack oak	97	1.3	1.4	2.7	16.8
Oak-hickory	234	.8	1.1	1.9	8.9
Total woods	414	1.1	1.3	2.4	22.4
Other	30	.3	.7	1.0	3.4
Average	177.8	1.1	1.4	2.4	19.2

1/ A sample is the actual number of ticks collected per 25 $yd.^2$.

The edge of the prairie is shaded during part of the day. This cuts out part of the exposure to irradiation and allows a higher humidity in this area. Woodlots eliminate even more of the sun and keeps the humidity higher and the temperatures lower than the exposed prairie (Lancaster 1957). Openings encountered in these studies within woodlots were surrounded by trees that provided considerable shade. Such woodlot openings had more ground vegetation than the surrounding woods.

Vegetative Density Study

A study conducted on the relationship between understory vegetation within a woodlot and lone star tick populations (Table II) showed that the higher nymphal populations were found where density of vegetation was low, while the higher adult populations were found where vegetation was dense.

TABLE II

COMPARISON OF THE NUMBERS OF TICKS PER[1/] SAMPLE WITH THE DENSITY OF UNDERGROWTH IN WOODLOTS IN COOKSON HILLS STATE GAME REFUGE DURING JUNE AND JULY, 1969

Density	No. of Samples	Males	Females	Adults	Nymphs
Less than 25% coverage	200	1.0	1.2	2.2	26.0
25-75% coverage	190	1.1	1.3	2.4	20.4
More than 75% coverage	48	1.4	1.7	3.1	13.4

1/ A sample is an estimate of the number of ticks in a 25 yd.^{2} area.

Table II shows that nymphs and adults are influenced differently by various amounts of understory vegetation. Higher adult populations found during 1969 in the thick vegetative cover were probably due to the occurrence of and utilization by many hosts in this area during the period of high nymphal populations the previous year. The high concentrations of nymphs were due to the presence of the host in areas of thin ground cover during the larval season (July and August) in 1968. The transition zone and the openings in the woods (Table I), where the vegetation is dense, have higher adult to nymph ratios than the woods.

Effect of Leaf Litter Cover on Tick Populations in Woodlots

The highest nymphal populations were found in areas where the litter was medium or dense, while the highest adult populations were found where the litter was thin, but the vegetative cover was high (Table III).

TABLE III

COMPARISON OF TICK DENSITY WITH THE DENSITY OF LEAF LITTER IN WOODLOTS IN COOKSON HILLS STATE GAME REFUGE, CHEROKEE CO., OKLAHOMA DURING JUNE AND JULY 1969

Amount of Litter	No. of Samples	Males	Females	Adults	Nymphs
Less than 25% coverage	47	1.9	2.0	3.9	13.5
25-75% coverage	188	1.1	1.2	2.3	30.6
More than 75% coverage	110	.8	1.0	1.8	27.1

The Effect of Shading on Tick Numbers

The north side of the woods averaged more than twice as many nymphs per sample as the other three sides (Table IV). The south side of the woods had fewer adults per sample than the other three sides. The north edge of the woods had more adult and nymphal ticks per sample than either of the other cardinal directions. The reason for this distribution is probably due to the greater amount of shade on the north side of the woods which allows a higher humidity over a period of time, and therefore, provides a more favorable habitat for the tick and its hosts. The east and west edges of the woods were somewhat more favorable than the south edge because they are shaded part of the day. The south edge is exposed to direct sunlight most of the day.

TABLE IV

EFFECT OF SHADING ON TICK NUMBERS IN THE TRANSITIONAL ZONE BASED ON THE DIRECTION FROM WOODLOTS DURING JUNE AND JULY, 1969 IN COOKSON HILLS STATE GAME REFUGE

Direction	No. of Samples	Males	Females	Adults	Nymphs
East	32	.9	1.4	2.3	6.5
South	62	.8	1.0	1.8	5.5
West	30	1.5	1.7	3.2	5.4
North	52	1.7	2.2	3.9	13.6

Relationship of Tick Populations to Height of Vegetation

A study of _A_. _americanum_ adult and nymphal populations in six major vegetation types according to height (Figure 2) showed that the adults and nymphs were most abundant in low deciduous trees. The lowest nymphal and adult populations were found in low grass and medium grass. Tall trees also had a low adult population.

Effect of Distance from Woodlots on Tick Populations

A study of the tick populations found at several distances from the woodlot-prairie ecotone showed that populations decreased with increased distance from the ecotone (Figure 3). This decrease in population is probably related to the habits of the tick's host and the season of the year that this habitat is used most frequently by such hosts.

Aggregation Study of Area No. 1

In the studies in Cookson Hills Refuge 1011 samples were taken. The upper 1% of the samples (1% of the area sampled) contained 41.6% of the total ticks, 46.5% of the total nymphs, and 22.6% of the total adults (Table V). The adults were more evenly distributed than the nymphs due to several large samples of nymphs. The number of nymphs per sample ranged from 0-1540 with an average of 12.21 nymphs per sample, while the number of adults per sample ranged from 0-86 with an average of 1.78 adults per sample.

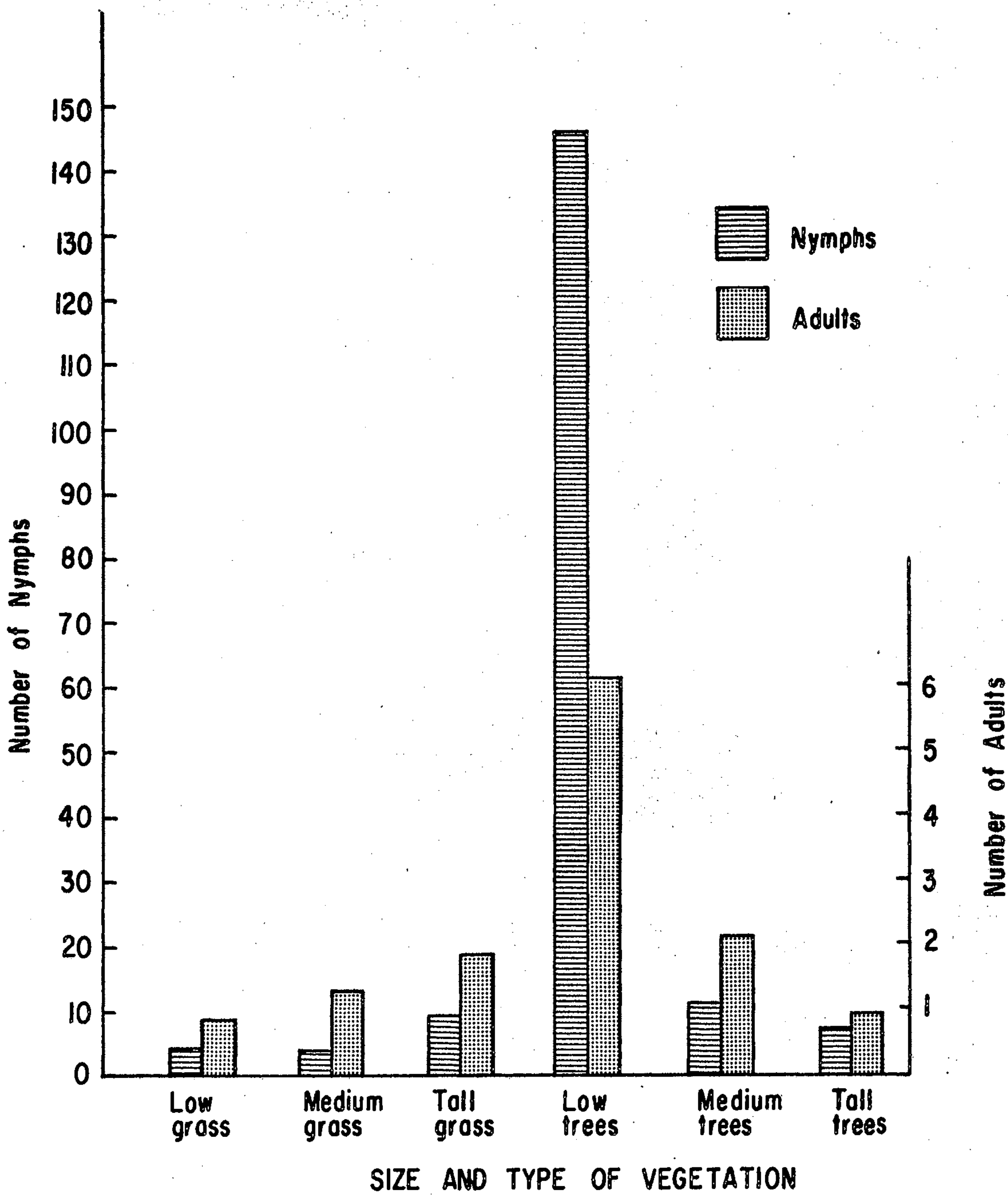

Figure 2. Average Number of Ticks Collected from Different Vegetation Types According to Height in Cookson Hills State Game Refuge, Oklahoma during June and July, 1969.

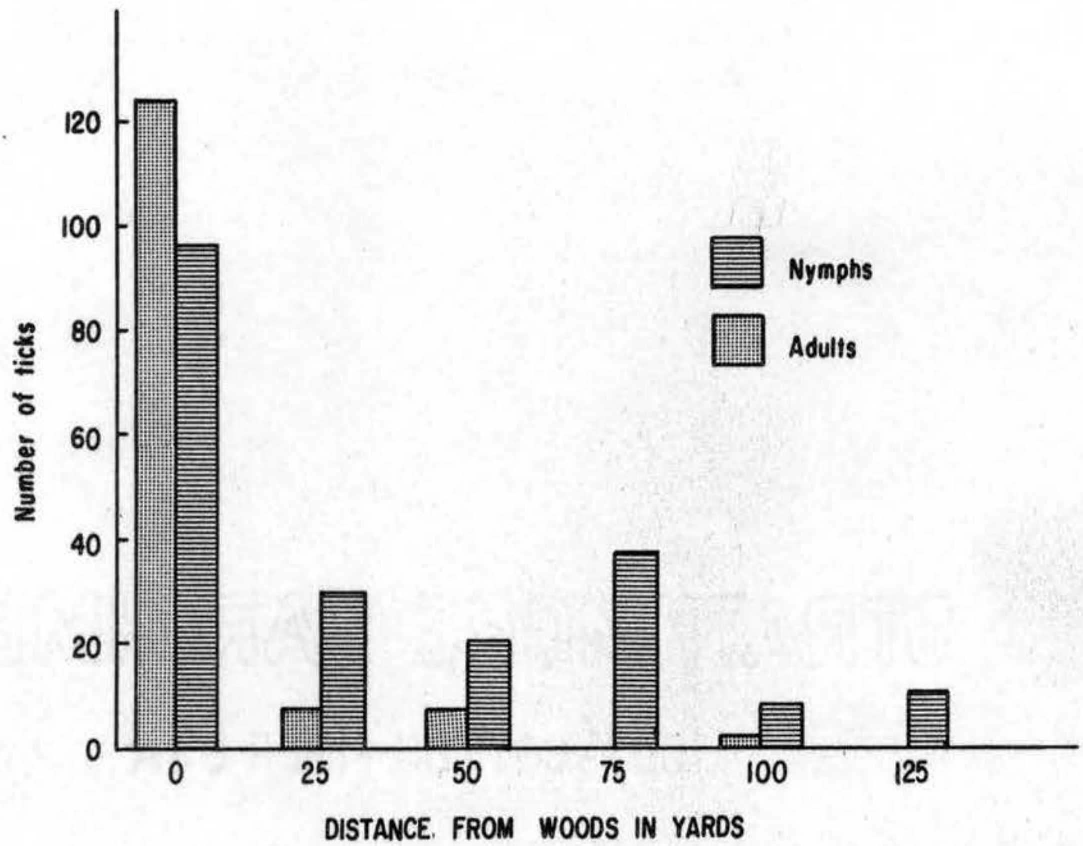

Figure 3. The Total A. americanum Sampled in a Prairie at 25 Yard Intervals from the Forest Edge in Cookson Hills State Game Refuge, Oklahoma during June and July, 1969.

TABLE V

PERCENTAGE OF THE TOTAL NUMBER OF TICKS COLLECTED FROM THE TOP 1, 5 AND 10 PERCENT OF THE TOTAL AREA SAMPLED DURING JUNE AND JULY 1969 IN COOKSON HILLS STATE GAME REFUGE, OKLAHOMA

Stage	Percent of Area		
	1%	5%	10%
Adults	22.6	51.0	76.6
Nymphs	46.5	66.9	76.8
Total Ticks	41.6	62.6	73.0

Population Study of Area No. 1

Extensive sampling of Study Area No. 1 made it possible to estimate its population during two periods, June 15 - June 18, and July 14-16. An estimate of the population during the rest of the year was made by plotting the ratio of the population with the seasonal distribution data collected from plots near the study area.

A significant drop in the tick populations occurred during the survey (Table VI). The nymph population dropped 60% between June 15 and July 15, 1969, while the adult population dropped 93% over the same period. It is estimated, using Figure 4, that the adult populations for the 60 acre study area reached a peak of 80,000 active adults on May 26, while the nymphs peaked at 730,000 on June 5, 1969. These estimates are probably low because over 7000 ticks were collected in a CO_2 trap in a single afternoon in this area on July 1.

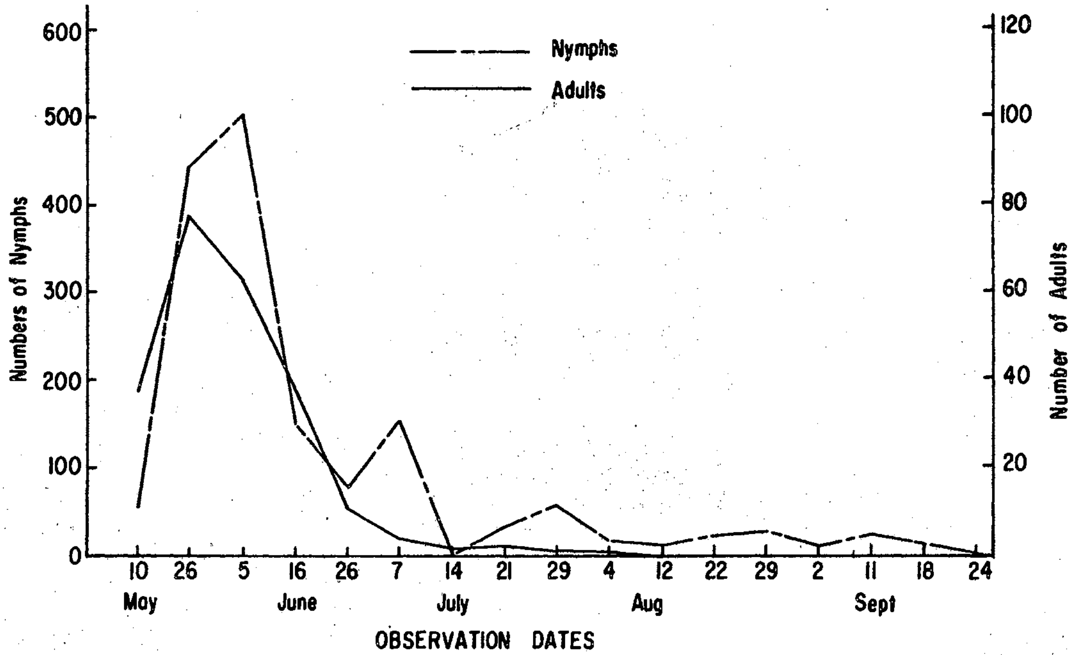

Figure 4. Seasonal Distribution of Adult and Nymphal Lone Star Ticks in Cookson Hills State Game Refuge, Oklahoma during June and July, 1969.

TABLE VI

ESTIMATE OF THE ACTIVE ADULT AND NYMPHAL A. AMERICANUM POPULATIONS IN STUDY AREA NUMBER 1 DURING JUNE AND JULY 1969 IN COOKSON HILLS STATE GAME REFUGE

Date	# of Samples	Adults/ Sample	Adults/ Acre[1]	Nymphs/ Sample	Nymphs/ Acre
June 15-18	282	3.4	652.8	19.2	3724.3
July 14-16	314	.3	54.8	7.6	1465.6

1/ One acre = 193.6

A. americanum Larval Movement Studies

Studies on the movement of *A. americanum* larvae during August and September 1968 showed that they tended to stay on the side of the plant opposite the light source, the wind and the rain, even in shaded woods. On fair days when the sun was in the southeast, the masses of larvae tended to be on the northwest and west sides of the stems and leaves (Table VII and Figure 5), while during the same time on cloudy days, the ticks showed less preference to be opposite the light source. This could be due to the larvae remaining in the same location they had assumed the day before while the sun was in the west. When the sun was in the southwest, many of the ticks had moved to the northeast, and by 7-9 p.m. most of the ticks had moved to the east side of the plant.

Occasionally during the study, whole masses of ticks disappeared from the plants due to wind, heat, rain, or animal disturbance. After

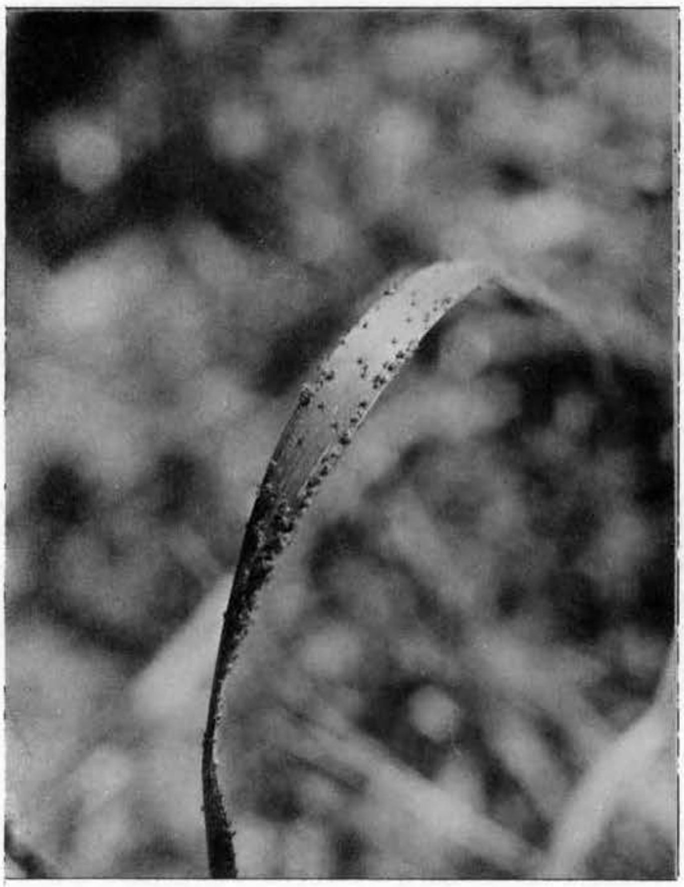

Figure 5. A Mass of A. americanum Larvae on a Blade of Grass Showing the Tendency to Clump on the Plant Away from the Impenging Sun Rays.

the ticks fell from their resting place, the size of the regrouped mass was reduced. Sometimes the ticks climbed the same plant, but frequently they chose lower plants. This dislodgement of ticks from their large masses could cause increased mortality, because the aggregation of larvae help prevent their desiccation.

TABLE VII

THE RELATIONSHIP OF THE OBSERVED LOCATION OF A. AMERICANUM LARVAE ON PLANTS TO THE DIRECTION OF THE SUN IN COOKSON HILLS STATE GAME REFUGE DURING AUGUST AND SEPTEMBER 1968

Time	Direction of Sun	Weather Conditions	Number of Observations Per Location									
			AB[1/]	BL[2/]	N[3/]	NE	E	SE	S	SW	W	NW
9-11	Southeast	Clear	3	8	1	1	1	0	0	0	7	4
9-11	---	Cloudy	5	14	0	3	0	0	5	0	2	0
9-11	---	Rainy	1	7	0	0	4	0	0	0	0	0
11-1	South	Fair	0	2	3	0	0	2	2	0	0	0
1-3	South-SW	Fair	5	25	3	16	8	3	1	2	0	0
3-5	Southwest	Fair	3	14	2	9	2	0	0	0	0	0
5-7	West-SW	Fair	2	6	0	7	9	0	2	1	0	0
5-7	---	Cloudy	5	4	0	0	0	0	2	0	0	0
7-9	---	Fair	3	9	0	2	7	0	1	1	0	0

1/ Above leaf 2/ Below leaf 3/ North side of leaf

Lees (1948) suggests adult I. ricinus move up and down the vegetation to regulate the body moisture content. He stated that a tick with adequate body moisture tended to climb to the tips of grasses where the humidity was lower. After a period of unsuccessful waiting, the body became desiccated and the tick returned to the mat at the base of the plants to replentish its body water. Wilkinson (1953) found that in Australian B. microplus, the main stimulus governing ascent of the grass blades, appeared to be positive phototaxis rather than negative geotropism to moderate light intensities.

Height of A. americanum on Plants

Adult A. americanum were found from the ground level up to 5 ft. above the ground on some plants. Nymphs were found from the ground to 4½ ft. and larvae ranged from ground level to 3½ ft. Wilkinson (1953) found that Boophilus larvae in Australia would climb over 9 ft. up a pole. Marikovskii (1945), while studying three species of Russian ticks, found them up to 2 m above the ground, but most ticks were found between 40 and 80 cm.

Dominant Ground Plant Species in Woodlots in Relation to Tick Populations

Desmodium spp., S. orbiculatus, A. virginicus, and Panicum spp. were the most common dominant plant species on the forest floor within the study areas. The highest nymphal populations were found in areas where S. orbiculatus was the dominant vegetation (Table VIII), while A. virginicus also harbored high nymphal populations. The highest

TABLE VIII

AVERAGE NUMBER OF TICKS PER SAMPLE OF THE DOMINANT FLOOR PLANTS IN WOODLOTS IN COOKSON HILLS STATE GAME REFUGE DURING JUNE AND JULY 1969

Plant	# of Samples	Males	Females	Adults	Nymphs
Broomsedge * (Andropogon virginicus)	50	2.7	2.7	5.4	22.0
Buckbrush * (Symphoricarpos orbiculatus)	68	1.3	1.4	2.7	38.5
Tick Clover * (Desmodium spp.)	31	3.2	4.6	7.8	8.9
Broad-leaf unolia (Unolia latifolia)	34	.2	.5	.7	3.0
Panicum spp.	19	.7	.8	1.5	7.0
Saw greenbrier * (Smilax Bona-nox)	12	.8	.7	1.5	15.8
Panicum anceps	10	.4	.3	.7	16.2
Sericea lespedeza * (Lespedeza cunata)	10	.3	.8	1.1	14.1
Wild rye (Elymus spp.)	4	2.2	1.5	3.7	6.0
Johnsongrass * (Sorghum halepense)	3	2.3	2.3	4.6	17.7
Blackberry (Rhubus spp.)	2	6.5	6.5	13.0	18.5

* Plants used in the laboratory plant preference study.

adult populations were found where Desmodium spp. was dominant, with A. virginicus also yielding high adult populations. The tick clover (Desmodium spp.) in this area was heavily browsed by deer, but A. virginicus was a poor food plant, seldom grazed by deer or cattle. Symphoricarpos orbiculatus provided a little browse. All three plant species mentioned here were found in areas frequented by deer and cattle.

Plant Preference Studies

Field Observations

The plant preference study was divided into two categories: observations of A. americanum on plants in the field, and a tick preference study of selected plants on a turntable.

In the field, Elymus spp. had the highest number of observed A. americanum while tick clover (Desmodium spp.), dead saw greenbrier (S. Bona-nox), broomsedge (A. virginicus), Panicum spp., and Bromus spp. also had high numbers of ticks associated with them (Figure 6). Many of these observations were made in areas with very high tick populations. The area that had the heaviest infestation was treated with a broad-leaf specific herbicide during 1967 and 1968, so the main plants in the area were grasses, mainly Elymus spp. This area was a bedding ground for cattle during 1967, causing high nymphal population during 1968.

Turntable Test

A factorial analysis was made of each of the 20 experiments conducted. Of the 20-three plant species comparisons, only three showed

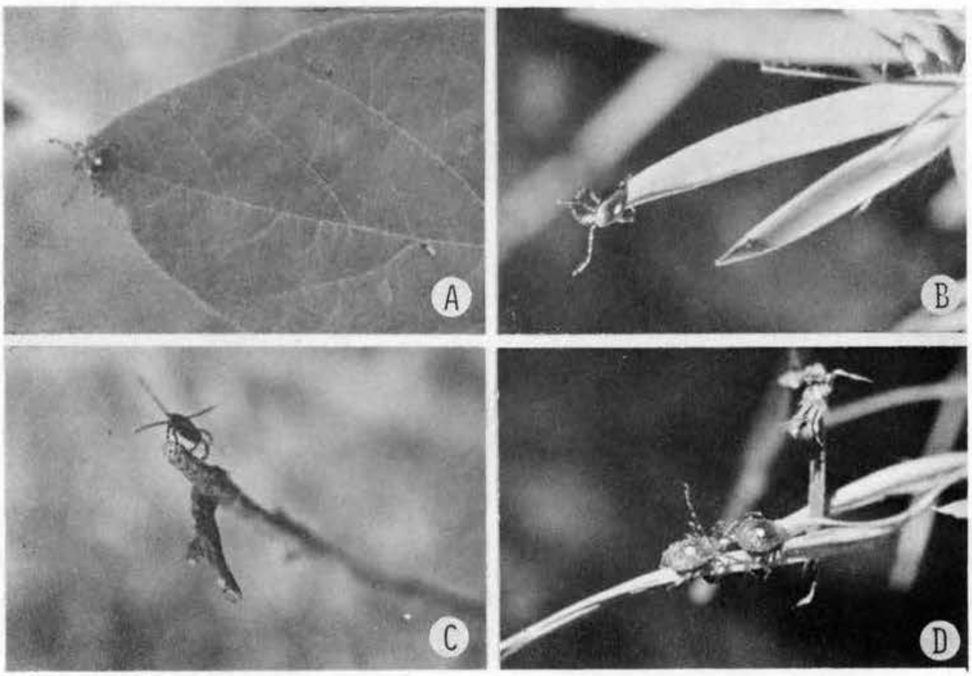

Figure 6. A. americanum Awaiting a Host on Four Plant Species Under Natural Conditions. A, Desmodium sp.; B, Panicum sp.; C, Symphoricarpos orbiculatus Twig; D, Andropogon virginicus.

fewer ticks hatched as the relative humidity decreased. Generally, a microclimate with a high humidity can be found around green plants because of transpiration. This enables the tick to climb the plant so it can come in contact with its host more readily, and still be in a zone of high humidity. As the air humidity decreases, the transpiration rate increases, keeping the humidity near the plant higher than the air humidity.

A. americanum are attracted to high concentrations of carbon dioxide (Hair 1969, personal communication). Observations in areas of high concentrations of ticks showed that tremendous numbers were attracted to a dry ice trap. Garcia (1962) found that D. occidentalis Marx. were attracted at a rate of 0-15 per hour to an outlet emitting carbon dioxide gas. Plants with higher respiration rates may be more attractive to A. americanum. Field and laboratory observations followed these assumptions fairly closely except for one species, dead A. virginicus.

Smith, et al. (1946), while studying D. variabilis, stated that they seemed to be attracted by animal scents to certain locations. This could explain why one species of plant was chosen more often than another. The plants with the higher attractance could retain animal odors longer than those of lower attractance. Desmodium spp., L. cuneata, and S. orbiculatus, the plants that attracted the most ticks in the tests, had pubescence over the leaves or stems, which probably retain animal odors readily. The plants attracting fewer ticks, S. Bona-nox and S. halepense, had smooth waxy covers that probably shed animal odors.

Studies on Overwintering A. americanum

A total of 232 soil samples were taken during this study in an effort to demonstrate the overwintering behavior of A. americanum. From these samples, 4 males, 6 females, and 10 nymphs were recovered, all were A. americanum. A few more ticks were taken from the samples of the undecomposed leaves (top) portion of the samples than from the soil beneath the leaves (Table IX). No larvae were found in the samples taken beneath plants that had masses of larvae on them the summer before.

MacLeod (1938) suggests that I. ricinus in Scotland moved to the base of the plants when the temperature becomes too low for them. There they overwintered until the temperatures became favorable for them to seek a host again. He stated that ticks inactivated by cold responded immediately to a rise in temperature, so an occasional tick could be found on animals in the winter as a result of activation by the host lying on them, or by a transient rise in air temperature. A. americanum apparently acts in a similar manner, becoming inactive as the temperature falls, then active as it rises again.

Zimina, et al. (1965) found 3 Hyalomma plumbeum (Panzer) females and 2 males of D. daghestanicus (Ol. 1929) in a m^2 soil sample. Amblyomma americanum nymphs and adults apparently crawl into cracks in the soil, rocks and/or logs and under leaves as cold temperature approaches and some may chose these places in the engorged state and then molt.

TABLE IX

NUMBER OF *A. AMERICANUM* TICKS COLLECTED AT TWO SOIL LEVELS DURING WINTER AND SPRING 1969 IN COOKSON HILLS STATE GAME REFUGE

Date	Level	# of Samples	# of Ticks Males	Females	Nymphs
Feb. 1, 1969	Top	24	0	0	1
	Bottom	24	0	0	0
	Both	24	0	1	2
Feb. 10, 1969	Top	20	0	2	2
	Bottom	20	1	0	3
Feb. 28, 1969	Top	20	1	0	0
	Bottom	20	0	1	0
Mar. 26, 1969	Top	20	0	1	2
	Bottom	20	0	1	0
April 14, 1969	Top	20	0	1	0
	Bottom	20	1	0	0
Top Total		104	2	2	3
Bottom Total		104	2	2	3
Other		24	1	0	1
Grand Total		232	4	6	10

CHAPTER V

SUMMARY AND CONCLUSIONS

A study was conducted in Cookson Hills State Game Refuge to determine the type of habitat preferred by _A. americanum_ (L.), the lone star tick. Samples were taken in 10 different habitat study areas between June 14 and July 15, 1969. A sweep net and a drag (3 x 4 ft. piece of muslin) were used to take the samples. The drag was pulled over the ground behind the person sampling, while he took a sweep per step with the sweep net for 25 steps. The numbers of each stage of tick on the drag and sweep were counted and recorded separately. Each sample was an estimate of the number of active _A. americanum_ in approximately a 25 sq. yd. area. Each 25 sq. yd. sample area was studied to determine the vegetative type, the dominant plants, the vegetation density, the density of the leaf litter, and the height of the plants.

Amblyomma americanum nymphal populations were highest in communities of sassafras, persimmon, and winged elm, followed in order by: openings in woods having grasses, legumes, and buckbrush; post oak-blackjack communities; oak-hickory communities; the forest-prairie ecotone; and the open prairie.

Studies on the effect of ground vegetation density and the leaf litter cover showed that adult and nymphal preferences varied. The highest populations of nymphs were found in areas where there was less than 25% coverage by ground vegetation, while the highest populations of adults were found in areas where there was more than 75% ground coverage by vegetation. The highest number of nymphs were taken from areas with 25-75% coverage by leaf litter, while the highest adult populations were found in areas in woods with less than 25% coverage by litter.

Shading had some effect on tick populations. Both adults and nymphs were most abundant on the north edges of woods, while they were least abundant on the south and west edges of the woods.

The height of the vegetation had a definite effect on the tick populations. The highest numbers of adults and nymphs per sample were found in low brushy trees, followed in order by medium trees and tall grass. The lowest number of adults were found in a low grass and tall trees, while the fewer nymphs were found in low and medium grasses.

Dominant ground plants in and around the woods with high tick populations include A. virginicus and S. orbiculatus with high numbers of nymphs and Desmodium spp. with high numbers of adults.

The tick population of a meadow decreased with increased distance from the forest-prairie ecotone. Two-fifths of the total ticks were taken in 1% of the samples, while three-fourths of them were taken in 10% of the samples.

Based on the sampling procedure used, an estimated 650 adults and 3724 nymphs per acre occurred in a 60 acre study area in mid-June. By mid-July the population had decreased to an estimated 55 active adults and 1465 active nymphs per acre.

Observations on A. americanum larvae on plants showed that, though usually shaded by trees, they normally chose the side of the plant opposite the sun or the rain.

During the winter of 1968-69, a total of 4 males, 6 females, and 10 nymphs of A. americanum were collected from 232 soil samples taken in areas of high tick populations during the summer of 1968 in Cookson Hills State Game Refuge. No larvae were collected. About half of the ticks were taken from leaf litter and the other half of the ticks were taken from the upper 1½ inches of top soil.

A factorial experiment was set up to test six plant species for tick preference. The plants were tested three species at a time using a turntable to equalize the light, temperature, and humidity. Of 20 test runs, only three showed significant differences between the plant species.

The tick habitat study showed that the majority of the ticks were found in areas most often frequented by their important hosts. Humidity and temperature also determine their distribution.

APPENDIX

MEAN NUMBER OF *A. AMERICANUM* ADULTS CHOOSING EACH SPECIES OF PLANT/RUN IN PLANT PREFERENCE TEST ON A TURNTABLE IN THE LABORATORY

Test No. 1	$\bar{X}$
Lespedeza cuneata	0.79
Andropogon virginicus	0.46
Smilax Bona-nox	0.58

Test No. 2	$\bar{X}$
Lespedeza cuneata	1.21
Andropogon virginicus	1.04
Sorghum halepense	0.67

Test No. 3	$\bar{X}$
Lespedeza cuneata	0.71
Andropogon virginicus	0.58
Desmodium spp.	0.29

Test No. 4	$\bar{X}$
Lespedeza cuneata	1.17
Smilax Bona-nox	0.96
Sorghum halepense	0.38

Test No. 5	$\bar{X}$
Lespedeza cuneata	0.58
Symphoricarpos orbiculatus	0.75
Andropogon virginicus	0.63

Test No. 6	$\bar{X}$
Sorghum halepense	0.33
Andropogon virginicus	0.63
Symphoricarpos orbiculatus	0.67

Test No. 7	$\bar{X}$
Lespedeza cuneata	1.33
Smilax Bona-nox	0.42
Symphoricarpos orbiculatus	0.71

Test No. 8	$\bar{X}$
Lespedeza cuneata	1.83
Smilax Bona-nox	0.92
Desmodium spp.	1.50

Test No. 9	$\bar{X}$
Lespedeza cuneata	1.04
Sorghum halepense	1.00
Symphoricarpos orbiculatus	1.67

Test No. 10	$\bar{X}$
Lespedeza cuneata	1.04
Sorghum halepense	0.33
Desmodium spp.	0.63

Test No. 11	$\bar{X}$
Lespedeza cuneata	1.29
Symphoricarpos orbiculatus	0.71
Desmodium spp.	1.12

Test No. 12	$\bar{X}$
Andropogon virginicus	1.17
Smilax Bona-nox	0.83
Sorghum halepense	0.58

APPENDIX (Continued)

Test No. 13	$\bar{X}$
Andropogon virginicus	0.63
Smilax Bona-nox	0.42
Symphoricarpos orbiculatus	1.17
Test No. 14	
Andropogon virginicus	1.21
Smilax Bona-nox	1.46
Desmodium spp.	1.83
Test No. 15	
Andropogon virginicus	0.79
Symphoricarpos orbiculatus	0.67
Desmodium spp.	0.88
Test No. 16	
Smilax Bona-nox	0.58
Sorghum halepense	0.54
Symphoricarpos orbiculatus	1.25

Test No. 17	$\bar{X}$
Andropogon virginicus	0.88
Sorghum halepense	0.92
Desmodium spp.	1.21
Test No. 18	
Smilax Bona-nox	0.54
Sorghum halepense	0.46
Desmodium spp.	0.75
Test No. 19	
Smilax Bona-nox	0.38
Symphoricarpos orbiculatus	0.71
Desmodium spp.	0.75
Test No. 20	
Sorghum halepense	0.33
Symphoricarpos orbiculatus	0.67
Desmodium spp.	1.29

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