EVALUATION OF *IXODIPHAGUS TEXANUS* HOWARD FOR CONTROL OF *RHIPICEPHALUS SANGUINEUS* LATREILLE

By

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LATREILLE

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CHAPTER I

INTRODUCTION

The first hymenopterous parasitoid of ticks recorded, Ixodiphagus texanus Howard, was originally isolated by L. O. Howard in 1907. Since that time several attempts to gain biological control of ticks through parasitoid liberations have failed. The majority of previous attempts utilized a closely related specie of I. texanus, Hunterellus hookeri Howard. Researchers working for the Montana State Board of Entomology released over four million H. hookeri in Montana, Oregon, Idaho and Colorado from 1927-1933 in an attempt to control Dermacentor andersoni (Parker & Butler 1928, Cooley 1930, Kohls 1930, Cooley & Kohls 1934). Larrouse et al. (1928) liberated H. hookeri on Naushon Island, Massachusetts in an attempt to control Dermacentor variabilis. Releases of over 90,000 females of H. hookeri were made by Smith and Cole (1943) for control of D. variabilis on Martha's Vineyard, Massachusetts from 1937-1942. None of the previous attempts were successful in noticably reducing tick populations nor did any of these attempts include I. texanus as a biological control agent. Since the recent recovery of this encyrtid wasp from northeastern Oklahoma and subsequent colony establishment (Bowman 1979), steps were guided towards future mass releases of this parasitoid for tick control.

Because hymenopterous tick parasitoids have on a number of occasions been recovered from naturally occurring tick populations in nature

it would seem logical that these would hold some promise for use in a tick management program. By successfully establishing an effective population of this parasitoid in the environment, a biological control of one of the livestock industries most damaging economic pests might be possible. This study deals with a number of observations which were designed to better understand the possibilities of the use of this parasitoid in this capacity.

CHAPTER II

BIOLOGICAL OBSERVATIONS OF *IXODIPHAGUS TEXANUS* HOWARD

The general laboratory biology of *I. texanus* has been reported (Bowman 1979). However there was information yet unknown about the biology of the parasitoid that was prerequisite to the advance of releases in the southwestern United States.

Successful establishment of the parasitoid in this area is predicated upon its ability to survive the harsh climate of Oklahoma winters in numbers large enough to maintain or progress the previous years population and regenerate when tick activity resumes in the milder spring weather. Larson and Green (1938) reported that *I. texanus* were able to overwinter in engorged or unengorged immature stages of *Haemaphysalis leporis-palustris* Packard located in Minnesota. Larrouse (1928) reported successful overwintering and maintenance of a similar hymenopterous parasitoid, *Ixodiphagus caucurtei* (*Hunterellus hookeri* Howard) in Massachusetts under field conditions.

The parasitoid normally reproduces bisexually with mating occurring soon after emergence from the tick. When unfed tick larvae are parasitized by the wasp only a few or less often a single parasitoid will emerge (Bowman 1979). If these tick carcasses contained unfertilized females capable of parthenogenetic reproduction, other than that of arrhenotoky, it would seem likely that a larger population could be

generated from those parasitoids which emerge from the replete larvae to parasitize the next tick stage, the unfed nymph. Most of the parasitic Hymenoptera, including Encyrtidae, exhibit the arrhenotokous form of parthenogenesis (Doutt 1958, Clausen 1940). However, Ishii (1923) reported that virgin females of an encyrtid species, Microterys speciosis Ishii, produced only female progeny.

In an effort to delineate important biological aspects of I. texanus, tests were devised to determine which developmental stage of the tick provided the most suitable host for passing the winter. Also, observations were made on reproductive parthenogenesis in I. texanus in order to gauge field populations.

Materials and Methods

Generalized Procedures. Dermacentor variabilis ticks were used as the parasitoid host in this study because it was shown in laboratory tests to be the most suited species for I. texanus in terms of actual parasitism and numbers of parasitoids emerged per tick (Bowman 1979). The colony ticks used to propagate parasitoids were maintained similar to the reported procedure by Patrick and Hair (1975). In this procedure, larvae were fed on rabbit hosts restrained in wire cages, while confined in rearing tubs. Adult and nymphal ticks were fed on sheep in orthopedic stockinette cells placed over a shorn area of the animals skin. In this way large numbers of uniformly aged ticks could be reared. Colony maintenance of I. texanus followed the procedures outlined by Bowman (1979) in which groups of unengorged nymphs were exposed to parasitoids at an approximate 1:10 parasitoid:tick ratio for approximately 48 hours.

Exposed nymphs were then allowed to replete on sheep hosts. After tick detachment they were held in humidity chambers that maintained the relative humidity between 93.5% and 96%. These chambers were maintained at 26°C with a photo-phase of 14:10 light:dark. All tick samples retrieved and monitored for parasitism during the following observations were maintained under conditions similar to those stated above.

Ixodiphagus texanus Overwintering Behavior. In order to ascertain if I. texanus would overwinter in central Oklahoma and which stage was most successful, three immature stages, replete larvae, newly molted nymphs and engorged nymphs of D. variabilis, were tested in the following manner. Three thousand unfed larvae were allowed to replete on a rabbit host. On the day the engorged larvae dropped from the host the ticks were placed in 2.35 ml, 8.5 cm. diameter paper cartons, 1000 ticks per carton, and covered by a clear film of Handi-Wrap® Saran Wrap secured with a rubber band. Immediately after the replete larvae were placed in the paper carton, parasitoids were introduced into the container at a ratio of 1 parasitoid per 10 ticks. The ticks remained exposed for 24 hours to insure satisfactory time for parasitism. The replete larvae were then separated into groups of 500 and placed in 6 different outdoor arenas on 10 October, 1981. These ticks remained in the arenas throughout the winter and were retrieved as completely as possible on 16 March, 1981. These overwintered ticks were then allowed to engorge on a sheep host and monitored for parasitoid development.

The method of testing overwintering success in unfed nymphal ticks is described below. Within 5 days after their molting process was completed, 3000 D. variabilis unfed nymphs were placed inside paper cartons at the rate of 1000 nymphs per carton. These ticks were exposed

for 24 hours to parasitoids at a ratio of 1 parasitoid per 10 ticks. The ticks were separated into groups of 500 and placed in 6 different outdoor arenas on 10 October, 1980. After retrieval on 16 March, 1981, the ticks were allowed to engorge on a sheep host and monitored for parasitoid development.

Three hundred newly molted D. variabilis nymphs were exposed to I. texanus as described above and then allowed to feed to repletion on a sheep host. After dropping from the host the ticks were separated into groups of 100 and immediately placed in 3 different outdoor arenas. A release of 300 exposed replete nymphs was continued on a weekly basis from 25 September, 1980 through 13 November, 1980. Beginning on 20 November, 1980 until 1 June, 1981, samples of 10 engorged nymphs were retrieved from the arenas bi-weekly and held in a humidity chamber to more closely monitor when winter kill actually occurred, if at all.

The outdoor arenas were situated in Stillwater, Payne Co., Oklahoma. The study site was located in a grove of black locust, Robinia pseudoacacia, trees with an existing canopy cover of approximately 90%. The humus layer was approximately 1.9 cm above a clay-loam soil type. The arenas consisted of 10.16 cm. diameter Cresline DS ® white PVC pipe cut to 15.2 cm. length. The top of each arena was closed with a piece of organdy cloth secured by a rubber band. The arenas were driven 6.4 cm. into the ground leaving 8.9 cm. above ground. After the onset of dormancy in the ticks the top of each arena was removed to thoroughly present the ticks to the natural environment.

Parthenogenesis Test. Three thousand unfed larvae were placed in paper cartons, described above, at the rate of 500 larvae per carton. The top of each carton was enclosed with a clear film of Handi-Wrap® Saran Wrap secured with a rubber band. Parasitoids were introduced into each carton at a ratio of 1:10 parasitoid:tick. They were placed in a humidity chamber at approximately 95% R. H. and 24°-26.5°C for 24 hours to allow sufficient time for parasitism. After exposure to parasitoids, the tick larvae were allowed to engorge on rabbit hosts. The engorged larvae were held in the humidity chamber for 21 days. After that period of time the larvae which exhibited signs of parasitism were placed singly in 12 x 75 mm culture tubes and held until parasitoid emergence occurred. These isolated larvae were monitored closely during the emergence period and solitary emerged parasitoids were given access to 5 freshly fed D. variabilis nymphs immediately after emergence. These nymphs were monitored for parthenogenetic parasitoid development.

Results and Discussion

Ixodiphagus texanus Overwintering. The subsequent unfed nymphs from the parasitized replete larvae and the parasitized unfed nymphs were allowed to engorge on a sheep host. Data was collected on the number parasitized and the average number of parasitoids emerging per tick (See Table I). Of 1,415 unfed nymphs which were retrieved and allowed to engorge, 153 ticks molted and the balance, 1,262, were successfully parasitized. A sample group of 100 nymphs originally parasitized as replete larvae and 100 nymphs from the group parasitized as unfed nymphs were monitored for the average number of parasitoids emerging. The

TABLE I

OVERWINTERING BEHAVIOR OF
IXODIPHAGUS TEXANUS HOWARD
IN *DERMACENTOR VARIABILIS*
SAY TICKS, WINTER, 1980

Parameters Studied	Tick Stage Parasitized: Replete Larvae	Tick Stage Parasitized: Unfed Nymph
# ticks in field	3000	3000
# ticks recovered	524	891
# ticks parasitized	524	738
# ticks molt	0	153
$\bar{x}$ # parasitoids/tick	24	15

replete larvae group averaged 24 I. texanus emerging per tick and the unfed nymph group yielded 15 parasitoids per tick. Based upon these findings it seems likely that I. texanus is capable of overwintering in central Oklahoma without much difficulty if the parasitoid is established in a natural tick population. Results obtained from the overwintering replete nymphs showed that only the ticks placed in the arenas on November 13, 1980 held a large percentage of overwintering parasitoids. It seems unlikely that significant numbers of nymphs would engorge at such a late date in the tick season. Therefore, the replete nymphal stage seems less favorable for parasitoid overwintering.

Parthenogenesis test. Three thousand flat larvae were originally exposed for parasitism; 718 subsequently exhibited signs of parasitism and were isolated and monitored thereafter. From the 718 isolated replete larvae, 50 contained solitary I. texanus which, after emergence, were given access to 5 replete nymphs for possible parasitism. Of the 50 samples exposed and monitored, 3 contained parasitized nymphs. The parasitoids which emerged in these samples were examined and contained only male progeny. From these observations it was determined that arrhenotokous reproduction in I. texanus may occur under certain conditions.

The function of the male I. texanus is primarily to mate the female. This mating occurs immediately after the parasitoids emerge from the tick. Since there would be no females emerging from these ticks it would thus seem that these arrhenotokously produced males are of little value in the natural population dynamics of the parasitoid, and would probably be useless in a tick management scheme.

CHAPTER III

SUCCESS OF *IXODIPHAGUS TEXANUS* HOWARD FOR CONTROL OF *RHIPICEPHALUS SANGUINEUS* LATREILLE IN DOG KENNELS

Since population dynamics of *Rhipicephalus sanguineus* are little known or published accounts are not available for the southwestern region it was necessary to first delineate the general biological trends for this tick species in its natural surroundings. This type of information was required before a logical approach to release of *I. texanus* on a periodic basis could be made. These observations were therefore conducted over a period of 2 years. The first years' observations done in 1980 were primarily concerned with the general biological behavior of *R. sanguineus* in central Oklahoma, whereas at the beginning of the next year sequential releases of *I. texanus* were begun and its success monitored for the season.

In order to best determine the possibilities of *I. texanus* as a biological control agent for *R. sanguineus* two series of studies were done in 1981. The first of these 1981 studies involved observations of the parasitoid released under somewhat controlled and artificially managed systems at Stillwater, Oklahoma. The second phase of the observations involved the parasitoids being released in a naturally infested commercial kennel at Holdenville, Oklahoma.

Materials and Methods

Biological Observations. Some basic life cycle trends of R. sanguineus were monitored in a commercial kennel at Holdenville, Hughes County, in central Oklahoma during 1980. The kennel housed Canis familiaris breed greyhound and was known to have had a history of high R. sanguineus infestations on greyhound dogs. The number of dogs confined in the approximately 2 hectare outdoor kennel fluctuated between 11 and 30. The pens were constructed with wire fencing and steel pipe and each of the 7 pens measured approximately 5 meters wide by 100 meters long. Short vegetation consisting mainly of native pasture grasses and weeds covered the soil in the pens with the exception of the area immediately around the wood and corrugated iron constructed dog houses of which each pen contained one.

Whole body tick counts were made on dogs on a weekly basis beginning on 15 May through 3 October by which time tick activity subsided. The counting method consisted of carefully parting the hair over the dogs body with the fingers to locate any tick stage on the dog's body. From these data cyclic trends and preferred attachment sites of R. sanguineus on greyhounds in this situation were seen. Observations were also made on general tick behavior for use in later studies. These data were then used as reference information to better coordinate release methods for not only the Holdenville release but also the Stillwater studies described below. The I. texanus colony was maintained and propagated by the methods of Bowman (1979) which was briefly described in Chapter I.

In an effort to assess the value of I. texanus in controlling kennel tick infestations, 3 separate observations were monitored. The

first observation, the sequential release kennel, Stillwater, involved the release of parasitoids sequentially into a managed kennel situation in which the dogs were artificially infested. Another observation, the single release kennel, Stillwater, involved a single early season release of I. texanus into a kennel artificially infested as above. This system was monitored in an effort to determine if the parasitoid would maintain a population in the kennel throughout the season. The third study, the commercial kennel release, Holdenville, consisted of periodic releases of the parasitoid in a naturally occurring tick population at a commercial kennel.

Sequential Release Kennel, Stillwater. The outdoor enclosure consisted of 2 pens and each measured 2.5 meters wide by 6 meters long. The pens were constructed with a steel rod framework on a concrete footing and covered on the sides and top with welded wire. The floor was covered with sand and gravel and the top was covered with corrugated iron. Housing within each pen consisted of wooden boxes, 60 cm. x 60 cm. x 60 cm., with removable tops. A program of disease prevention and veterinary care was provided by the Oklahoma State University School of Veterinary Medicine. Four dogs, Canis familiaris breed Irish setters, were confined in the kennel, 2 males in 1 pen, 2 females in the other pen.

The 2 dogs in each pen were artificially infested with adult R. sanguineus from the laboratory colony in early May. The resultant larvae began attaching to the dogs in late June. The larval population was supplemented in both pens at that time with colony larvae to raise the initial larval infestation to a moderate number, approximately 500 larvae per dog.

Periodic releases of parasitoids were made in the kennels during periods of activity of susceptible tick stages e.g. unfed and fed larvae and nymphs. The actual liberation of parasitoids into the kennels began by releasing newly emerged parasitoids daily from the humidity chamber into the dog pens, dog houses and directly on the infested dogs hair. Later, due to insufficient parasitism, the release method was altered in an attempt to introduce an acclimatized parasitoid into the kennels. The alteration was by placement of parasitized ticks within a week of emergence in paper cartons which were left in or near the dog houses until emergence of parasitoids was complete.

Samples of ticks from the kennel were taken after each major release period to determine the percentage of parasitism. The collection of sample ticks was facilitated by fastening corrugated cardboard high along the interior walls of the dog houses. Replete ticks moved upwards into the corrugations of the cardboard for use as molting sites and could be easily and quickly collected by removing the outer layers of the cardboard and retrieving the ticks. Many samples contained replete larvae, unfed nymphs and replete nymphs. Replete larvae and unfed nymphs contained in samples were reared through the replete nymphal stage to check for parasitism due to the 'period of latency' described by Cooley and Kohls (1934) in which parasitoids do not develop when replete larvae are parasitized until the replete nymphal stage is reached.

Calculation of the number of parasitoids released during the test was done by randomly selecting a sample from the group of parasitized ticks to be released. This sample was kept in the laboratory and the

number of parasitoids which emerged was divided by the number of ticks with emergence holes in their carcass to obtain the average number of I. texanus emerged per tick. The average number was multiplied by the number of ticks in the release group which had emergence holes to calculate total field emergence for that release group. Ticks which were found attached to the dogs were recorded bi-weekly for the number and stage on that particular date. The tick counts were made by examining all external portions of the dogs body as described earlier.

Single Release Kennel, Stillwater. The outdoor enclosure was a single pen, 12.5 meters x 4.5 meters, constructed with a steel pipe framework on a solid concrete floor and covered on the sides with chain link fencing. The top was covered with corrugated iron and housing inside the pen was provided by a dog box similar to the dog boxes used in the sequential release kennel. Two male Irish setter dogs were confined to this facility for the duration of the study. These two dogs were artificially infested with R. sanguineus adults from the laboratory colony on 12 and 17 May, 1981. The subsequent larval attachment, which was first detected on 29 June was smaller than anticipated so laboratory larvae increased the population to a suitable infestation level of approximately 100 larvae attached per dog by 6 July.

A single mass release was made by liberating newly emerged parasitoids daily from the laboratory into the pen. This was done each day until the emergence of I. texanus from the group of ticks ceased. The parasitoids were allowed to disseminate into the dog pen from a holding carton placed beside the dog house.

The dogs were examined bi-weekly for tick attachment by the whole body count method already mentioned. After the release period samples of ticks from the kennel were taken during peak periods of activity throughout the tick season to determine if I. texanus were able to maintain a colony in this system. The collection of the tick samples was facilitated by the use of cardboard collecting devices mentioned above. Replete larvae and unfed nymphs were reared through the replete nymphal stage and monitored for parasitism.

Calculation of the number of parasitoids liberated in this observation corresponds to the method of calculation used in the sequential release test.

Commercial Kennel Release, Holdenville. The three pens selected as a release site were part of the commercial kennel described in the biological observations section. The pens were approximately 5 meters x 100 meters and situated in a grassy pasture. Two dogs, greyhound breed, were confined to each pen by a barrier of steel pipe and welded wire. A dog house constructed of wood with corrugated iron roofing was in each pen.

Parasitoid releases were made during periods of activity of susceptible stages by leaving cartons containing parasitized ticks within a week of emergence in the grass and weeds near the dog pens until emergence was complete.

The collection of ticks representing the sample from the kennel was done on a weekly basis during the observation period. Cardboard collecting devices similar to those used in the Stillwater kennels facilitated the collection of the sample.

The collected tick samples were monitored for parasitism in the

same manner as the tick samples retrieved from the Stillwater kennels. Calculation of the number of parasitoids released during this observation was also calculated by the method used for the Stillwater kennel observations.

Whole body tick counts were made on each dog on a weekly basis. This was done by the same method mentioned previously.

Results and Discussion

Biological Observations. In the established tick populations near Holdenville, in 1980, adult R. sanguineus ticks were active before 15 May. The larval activity began in late May and reached its highest level (53/dog) approximately two weeks later. These larvae were apparently the progeny of overwintering female ticks since they were the first immature ticks observed in the kennel. As indicated in Figure 1 the initial larval period and the long initial nymphal period which lasted from mid-June through mid-July should have provided ample time for parasitoid liberations during activity periods of susceptible tick stages.

It was also noted that there were two distinct generations of R. sanguineus during the summer. This was observed during the summer of 1980 at Holdenville as indicated on Figure 1 and further seen in the Stillwater studies the following summer, (Figure 2). An effective parasitoid population, if established in the infested system hopefully would have shown high levels of tick parasitism in samples retrieved during the second generation of the summer.

Observations of R. sanguineus preferred sites for attachment on

the greyhounds housed in the kennel at Holdenville during the summer of 1980 indicated that adult ticks preferred to attach in areas between toes, on ears, on the neck and on the middle back of the animal while immature stages showed a predilection to feed between the toes, under the belly, on the ears and between the hind legs of the dogs (Table II).

Observations of general tick behavior in the infested kennel revealed that all active stages were inclined to crawl upwards when not in pursuit of a host and many times these were seen crawling on the walls and ceilings or hiding in cracks of elevated structures which the tick may have chosen as a site for molting or ovipositon. This information became useful in devising a tick sampling device during the following studies.

Sequential Release Kennel, Stillwater. From 5 July through 15 July, 1981 the first release of I. texanus was made during the initial period of larval attachment. This release was made by releasing newly emerged adults daily from a site less than 60 cm. from the dog houses which invariably housed unfed and replete larval ticks during this release period. The dogs were inspected daily but due to the difficulty of counting attached larvae, it was estimated that the infestation averaged 250 larvae per dog through this period and it was not uncommon to see in excess of 1500 replete larvae in and around each house. On 13 July and average of 165 nymphs and 50 larvae were feeding on each dog, by 16 July the number of ticks had risen to 1200 nymphs and 10 larvae attached per dog. Figure 2 details the estimated total tick population on the dogs during the test which also gives some indication of the high number of ticks which had not attached

Figure 1. Rhipicephalus sanguineus Latreille seasonal distribution, Commercial Dog Kennel, Holdenville, Oklahoma, 1980

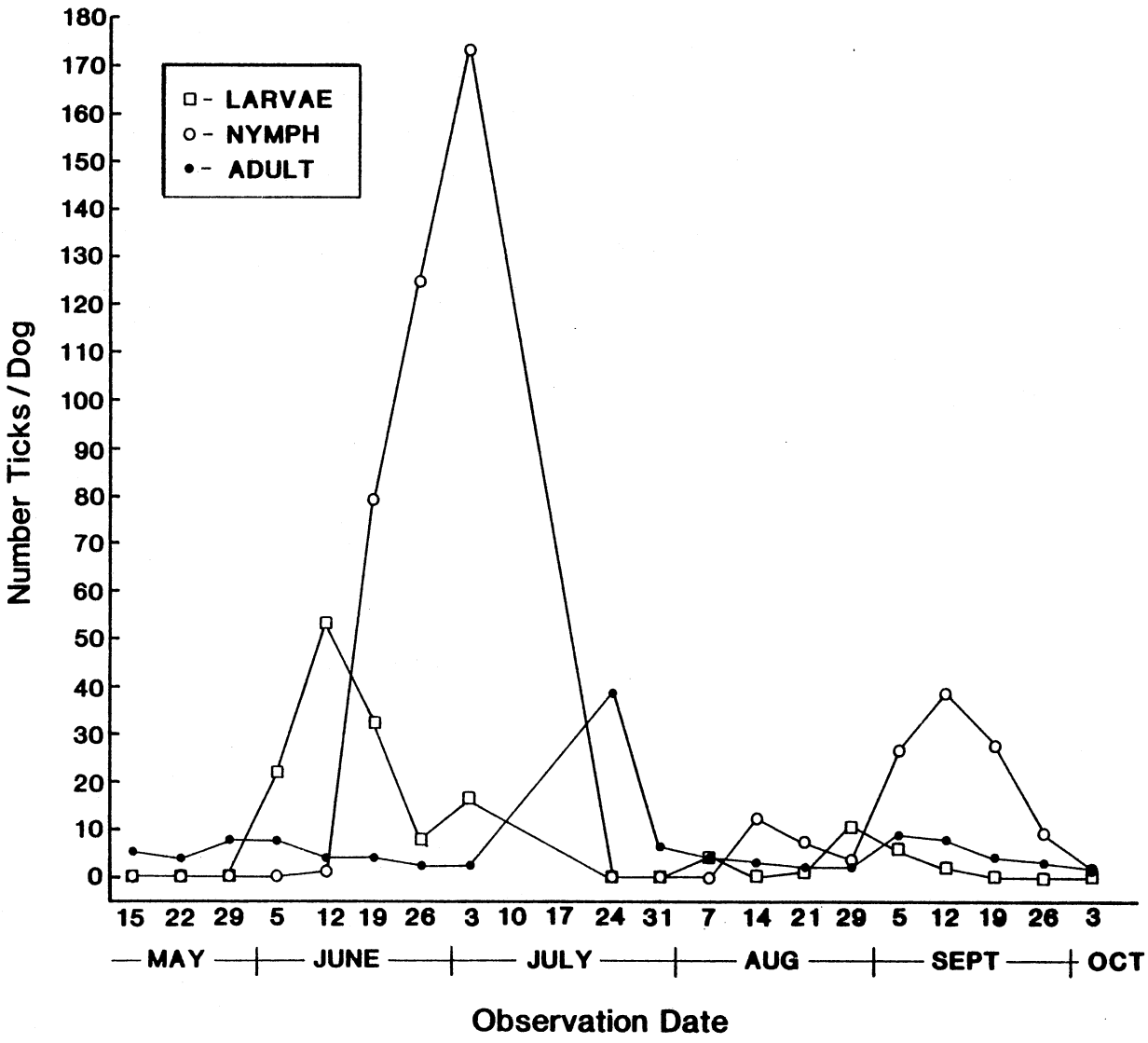
□ - LARVAE
○ - NYMPH
• - ADULT
Number Ticks / Dog
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
15 22 29 5 12 19 26 3 10 17 24 31 7 14 21 29 5 12 19 26 3
MAY
JUNE
JULY
AUG
SEPT
OCT
Observation Date

Figure 2. Rhipicephalus sanguineus Latreille seasonal distribution, Ixodiphagus texanus Howard release dates, Sequential Release Kennel, Stillwater, Oklahoma, 1981

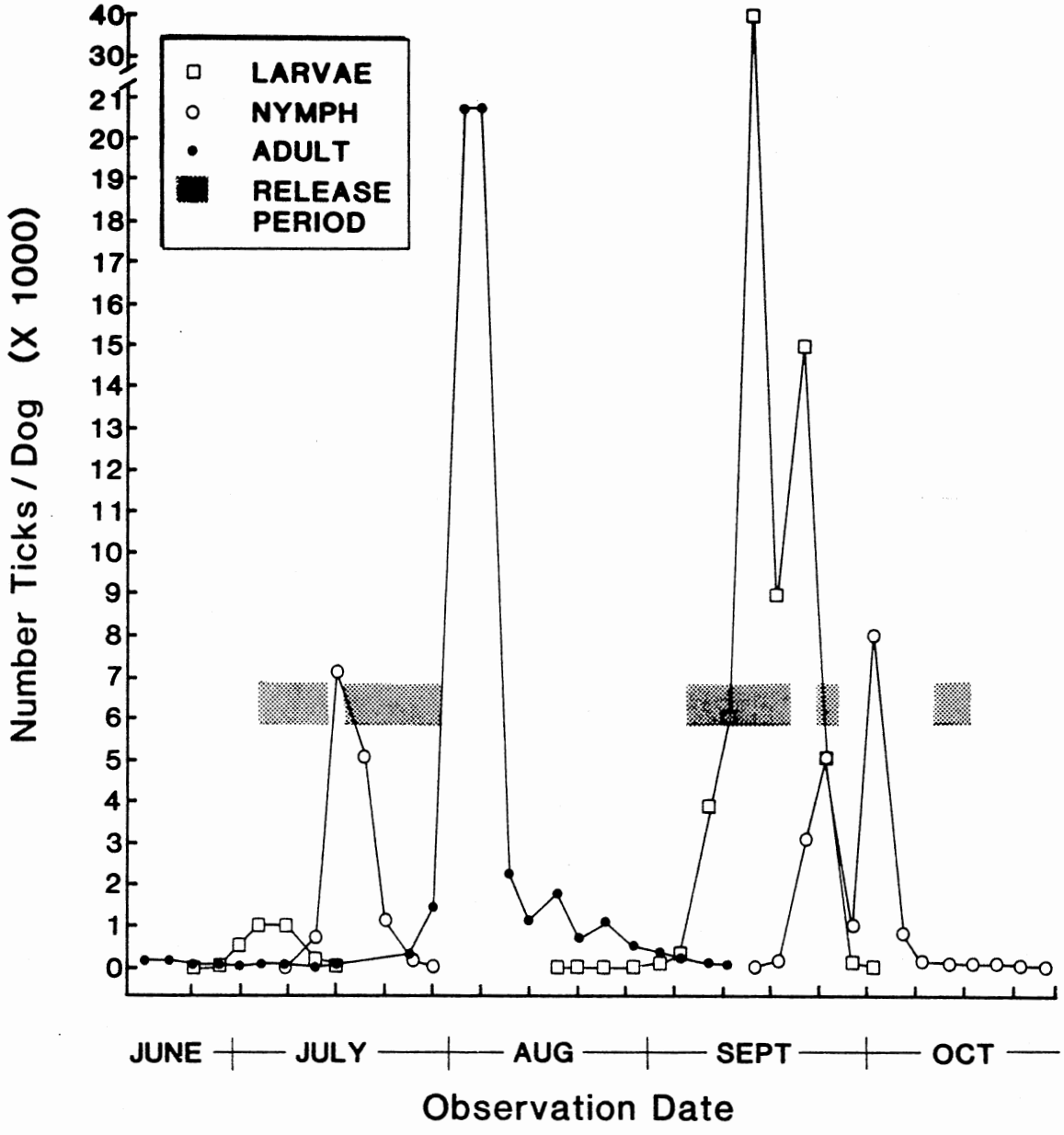
LARVAE
NYMPH
ADULT
RELEASE PERIOD
Number Ticks / Dog (X 1000)
40
30
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
JUNE
JULY
AUG
SEPT
OCT
Observation Date

TABLE II

PREFERRED ATTACHMENT SITES OF *RHIPICEPHALUS SANGUINEUS* LATREILLE ON GREYHOUND DOGS, HOLDENVILLE, OKLAHOMA, SUMMER, 1980

Location on Host	% of Total Ticks	
	Immature	Adults
between toes	33.20	35.64
under belly	27.22	3.58
on ears	25.21	24.70
between hind legs	13.22	1.19
on back of neck	0.22	12.24
lower forelegs	0.42	3.79
between front legs	0.45	2.71
middle back	0.01	5.85
throat area	0.01	5.20
upper front legs	0.01	3.14
sides (ribs)	0.02	0.99
head & face	0	0.54
lower back legs	0	0.43
lower back	0.01	0
TOTALS	100.00%	100.00%

or had already engorged and which were also in the vicinity available for parasitism. The Figure also has the dates underlined during which time parasitoids were either being released or emerging in the kennel. Table III indicates when parasitoid releases occurred, the number released and the number of parasitized ticks recovered from field samples. The number of I. texanus released from 5 July through 15 July was 12,555. Two days later, 17 July, another release began which lasted for 10 days. During that time an additional 29,090 parasitoids were released. From July 22-31 another group was released directly into the doghouse and on the dogs hair daily. This group totaled 11,872 I. texanus. Field samples were taken from the kennel on 15, 24 and 31 July. Of 6,851 ticks sampled, only 5 ticks were parasitized. The adult phase of the tick life cycle dominated the population attacking the dogs for virtually the entire month of August. By 28 August, larvae were again appearing on the dogs signalling the start of the second cycle of the tick season. The release of parasitoids during the remainder of the test consisted of leaving parasitized ticks, within a week of emergence, in paper cartons which were left near the dog houses until emergence was complete. The second larval period began 28 August and quickly grew to enormous proportions. By 15 September the average number of larvae attached per dog peaked in excess of 10,000 larvae. This larval stage remained abundant until the end of September. By that time, nymphal activity was increasing with over 1000 nymphs attached per dog. The nymphal activity peaked on 2 October at a number of approximately 8000 ticks per dog and remained high until 9 October. By 23 October tick activity had subsided for the season. From 5-20 September adult parasitoids were continuously

TABLE III

DATES AND NUMBERS OF IXODIPHAGUS TEXANUS HOWARD RELEASED, RHIPICEPHALUS SANGUINEUS LATREILLE SAMPLE SIZES AND NUMBERS OF R. SANGUINEUS LATREILLE PARASITIZED, SEQUENTIAL RELEASE KENNEL STILLWATER, OKLAHOMA, 1981

Date	I. texanus Released	R. sanguineus Sample Size	# Ticks Parasitized
July 5	12,555		
15		1,099	1
17	29,090		
22	11,872		
24		5,225	4
31		527	0
September 5	10,635		
10	25,963		
14	6,719		
15		8,057	11
23		3,398	46
24	677		
October 1		163	0
11	42,350		
21		120	3
Totals	139,861	18,589	65

emerging from parasitized ticks placed near the pens. During that period 43,317 I. texanus were released. A field sample of 8,057 ticks retrieved on 15 September held 11 parasitized ticks, another field sample of 3,398 ticks retrieved on 23 September held 46 parasitized ticks. From 24-27 September a release of 677 I. texanus was made. A subsequent field sample of 163 ticks retrieved on 1 October showed no parasitism. Near the end of the final nymphal phase, from 11-16 October, 42,350 parasitoids were liberated and the final field sample of 120 replete nymphs collected on 21 October contained 3 parasitized ticks.

Single Release Kennel, Stillwater. This test was conducted to determine if I. texanus would be capable of maintaining a colony of sufficient size to control a R. sanguineus infestation throughout the tick season from one large, early season release of the parasitoid. By 6 July the infestation level of R. sanguineus seemed suitable for parasitoid liberation, and from 6-16 July daily releases of adult I. texanus were made into the kennel. Adult parasitoids were allowed to disseminate at will from a holding carton placed beside the dog house. Before and after I. texanus releases, the developing parasitoids were held in a humidity chamber. By the end of the release period, 16 July, the larval activity had been replaced by the nymphal stage which averaged 78 nymphs per dog. Each day during the release period, replete larvae and/or nymphs were seen crawling about the surfaces of the dog house. A total of 24,690 I. texanus were released directly into the pen from 6-16 July. A sample of immature ticks was retrieved from the kennel on 15 July. The sample contained replete larvae, unfed nymphs, and replete nymphs. Of 365 ticks sampled on

15 July, 30 ticks were parasitized. Another sample of 141 ticks retrieved on 24 July held 6 parasitized ticks. A final sample was retrieved on 6 October during the last nymphal period. That sample of 99 replete nymphs showed no parasitism. Table IV indicates the dates and magnitudes of parasitoid releases, tick sample sizes and the number of sample ticks parasitized during this observation.

Commercial Kennel Release, Holdenville. In an effort to establish I. texanus in a natural occurring R. sanguineus kennel population, 3 weekly parasitoid releases were made in a commercial dog kennel during the first nymphal activity period. Releases were made by leaving cartons of parasitized ticks within a week of emergence in grass and weeds near the dog pens until emergence occurred. Tick counts were made on each dog weekly. On the first release date, 26 June, 1981, 2000 I. texanus were released. A field sample of 84 replete nymphs retrieved on 3 July showed no parasitism. On the second release date, 3 July, 3,895 parasitoids were released. A subsequent field sample of 144 replete nymphs retrieved on 14 July contained 1 parasitized tick. The third release of parasitoids was on 14 July, when 17,565 I. texanus were released into the kennel area. However, on 21 July, due to an increasing infestation of R. sanguineus adults, the dogs and their confinement facilities were sprayed with an acaracide forcing a premature termination of this test. Table V indicates the dates and magnitudes of parasitoid releases, tick sample sizes and the number of sample ticks parasitized during this observation.

TABLE IV

DATES AND NUMBERS OF IXODIPHAGUS TEXANUS HOWARD RELEASED, RHIPICEPHALUS SANGUINEUS LATREILLE SAMPLE SIZES AND NUMBERS OF R. SANGUINEUS LATREILLE PARASITIZED, SINGLE RELEASE KENNEL, STILLWATER, OKLAHOMA, 1981

Date	I. texanus Released	R. sanguineus Sample Size	# Ticks Parasitized
July 7	24,690		
15		365	30
24		141	6
October 6		99	0
Totals	24,690	605	36

TABLE V

DATES AND NUMBERS OF IXODIPHAGUS TEXANUS HOWARD RELEASED, RHIPICEPHALUS SANGUINEUS LATREILLE SAMPLE SIZES AND NUMBERS OF R. SANGUINEUS LATREILLE PARASITIZED, COMMERCIAL RELEASE KENNEL, HOLDENVILLE, OKLAHOMA, 1981

Date	I. texanus Released	R. sanguineus Sample Size	# Ticks Parasitized
June 26	2,000		
July 3	3,895	84	0
14*	17,565	144	1
Totals	23,460	228	1

* Observations were terminated on this date due to insecticidal treatment of the premises.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The objective of this study was to assess the possibility of utilizing Ixodiphagus texanus Howard as a biological control agent for the control of Rhipicephalus sanguineus Latreille in dog kennels.

Observations from I. texanus releases for R. sanguineus control in dog kennels showed that the parasitoid probably does not hold much potential for success in such applications. This was shown during the tick season of 1981 in three separate observations. The first observation was in the sequential release kennel at Stillwater, Oklahoma where 139,861 I. texanus were released into the R. sanguineus infested kennel. Field samples of 18,589 ticks taken during the same period contained only 65 parasitized ticks. The second observation in a single release kennel at Stillwater showed a slight amount of parasitism in two early season samples of field ticks but no parasitized ticks were recovered in a late season sample. The third observation was in a commercial kennel at Holdenville, Oklahoma. This observation was terminated early due to an increasing tick infestation, however, early observations indicated low parasitism in this situation also.

Some biological observations of I. texanus indicated that the parasitoid could probably overwinter in central Oklahoma with little difficulty if established in a natural tick population. Also it was

determined that an arrhenotokous type of parthenogenetic reproduction may occur in I. texanus under certain conditions, however the arrhenotokous male progeny would probably be of little value in a tick management scheme.

Biological observations of R. sanguineus during the 2 tick seasons monitored during the study indicated that there were 2 distinct generations of the tick during each season. It was also noted that adult ticks preferred to attach in areas between toes, on ears, on the neck and on the middle back of dogs while immature stages showed a preference to feed between the toes, under the belly, on the ears and between the hind legs of the dogs. Observations of general tick behavior revealed that all active stages were inclined to crawl upwards when not in pursuit of a host.

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