### HOST SUITABILITY AND LABORATORY BIOLOGY

OF IXODIPHAGUS TEXANUS HOWARD

By

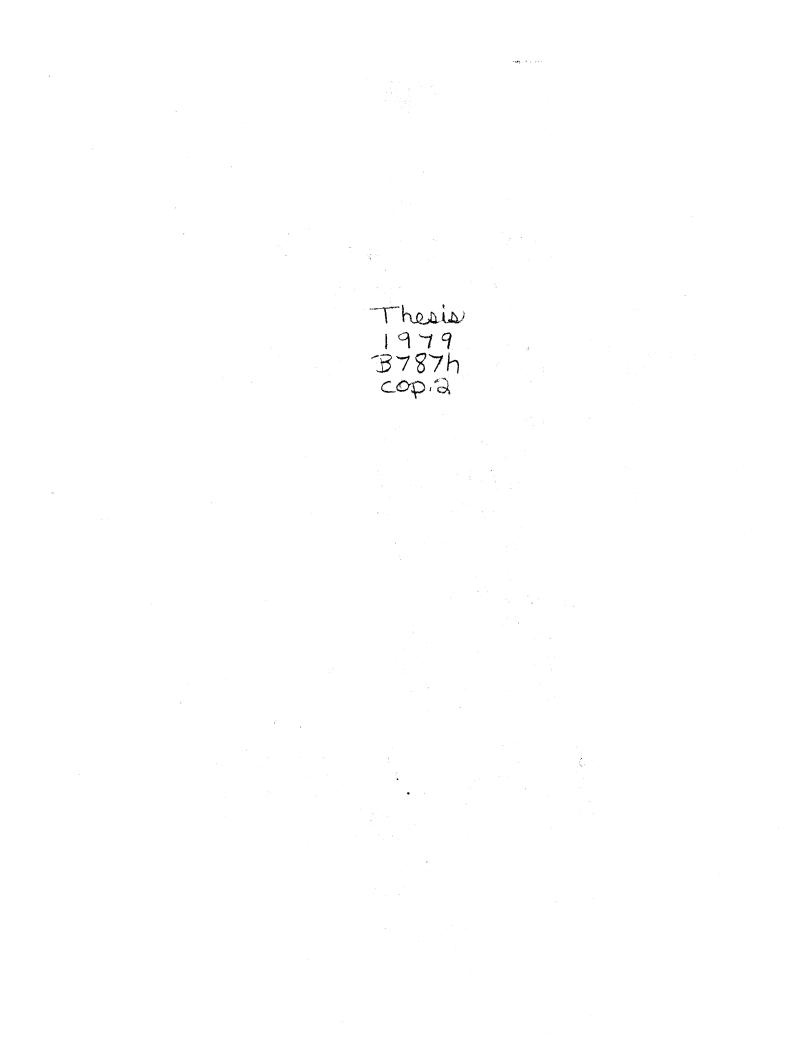
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### CHAPTER I

#### INTRODUCTION

In 1907 L. O. Howard described the first hymenopterous parasitoid of ticks, <u>Ixodiphagus texanus</u> Howard. Since that time 5 other species have been identified, <u>I. mysorensia</u> Mani, <u>I. biroi</u> Erdos, <u>I. hirtus</u> Nikol'skaya, <u>Hunterellus hookeri</u> Howard, and <u>H. theilerae</u> Fielder. The successful laboratory rearing of <u>H. hookeri</u> by Wood (1911) led to several attempts to mass release parasitoids for tick control in the late 1920's and early 30's, all of which proved unsuccessful. In the 4 decades following these early attempts on tick bio-control, little interest has been shown in the control of ticks by parasitoids.

In the past 50 years, millions of dollars have been spent on research efforts to control ticks, while we continue to suffer from many diseases transmitted by ticks, as well as experiencing great economic losses in the livestock industry and recreational areas (Williams 1977, Hair & Howell 1970). To compound this problem, the Environmental Protection Agency (EPA) has taken many of the effective acaricides off the market in an effort to reduce environmental pollution. Such happenings have encouraged the search for alternative control approaches such as effective biological controls. Early attempts to use hymenopterous parasitoids for biological control of ticks could have failed because of inadequate numbers of parasitoids being released compared to

the great geographical area covered by the releases. Morton (1928) released 381,190 parasitoids during the summer in the Bitter Root Valley and central and eastern Montana, Smith and Cole (1943) released an estimated 100,000 parasitoids on Martha's Vineyard Island, Massachusetts over a 3 year period, and W.H.O. (1965) refers to 4 million parasitoids released from 1927 to 1933 in Montana, Colorado, Idaho, and Oregon. As stated by Van Den Bosch (1973) it appears to be necessary to release large numbers of parasitoids in the same geographical area in a sustained release fashion to allow the maximum opportunity for successful establishment of these parasitoids.

Stacey (1976) reported the disappearance of a significant number of <u>Amblyomma maculatum</u> Koch, replete females which were released in open topped arenas in different prairie habitats in Pawnee County. Upon receiving this report, a preliminary survey of predators and parasites of replete female ticks was conducted (Bowman 1977).

This survey was conducted in the same area Stacey had reported the disappearance of replete ticks and was accomplished by glueing replete females to the pedal of a (McGill Can't Miss<sup>R</sup> mouse trap) and (Victor<sup>R</sup> rat traps) and glueing replete females to a size 14 fish hook which was firmly anchored by a 2 foot piece of nylon string. These traps were placed in the field and checked on a daily basis during the months of May, June and July of 1977. By using these techniques 7 predators of replete female ticks were incriminated in this survey. These include, rats (<u>Sigmodon</u> sp.), mice (<u>Peromyscus</u> sp.), quail (<u>Colinus virginianus</u>), box turtle (<u>Terrapene carolina</u>), oppossum (<u>Didelphis virginiana</u>), spiders (<u>Phalangidae</u> sp.) and Katydids (<u>Pseudophyllinae</u> sp.). However, during this survey there were 228

times in which traps were tripped without the animal being caught and in many cases the ticks were eaten or partially eaten indicating the presence of a predator.

This preliminary survey indicated a degree of biological control, however, the magnitude of tick control by predation cannot be determined by the information gained in this survey.

In May of 1978 a survey of biological tick control agents in Oklahoma was initiated which continued the search for additional predators of replete females as well as placing special emphasis on finding tick parasitoids by means of a field survey (Bowman 1978).

Although little additional information was obtained on tick predation, in August the survey produced 8 parasitized ticks, <u>Haemaphysalis</u> <u>leporis-palustris</u> (Packard) collected from Nowata County in northeastern Oklahoma. Parasitoids emerged from a replete larva and replete nymphs collected from quail (<u>Colinus virginianus</u>), sparrow (<u>Ammodramus</u> <u>savannarum</u>) and cotton-tail rabbit (<u>Sylvilagus floridanus</u>). Additional parasitoids emerged from 2 <u>H</u>. <u>leporis-palustris</u> collected from cottontail rabbits from the same area in September. The specimens were identified as <u>Ixodiphagus texanus</u> Howard by E. E. Grissell, Systematic Entomology Laboratory, II B III, U. S. Department of Agriculture.

From the naturally occurring population of <u>I</u>. <u>texanus</u> found in Nowata County in August 1978, the successful colonization of these parasitoids has now been achieved. In order to increase the efficiency of colony production for the purpose of possible future mass releases, it was necessary to have a greater understanding of the life history and biology of <u>I</u>. <u>texanus</u>. To accomplish this objective it was desirable to know basic facts such as the preferred tick host species, the preferred

life stage of the tick host, the most suitable tick host species for mass rearing, the most suitable life stage of the tick host for colony rearing, the average number of ticks successfully parasitized (adult parasites emerge) of each tick host species, the average mortality of each tick species due to parasitism, and the average number of parasitoids emerging from each tick species.

#### CHAPTER II

#### METHODS AND MATERIALS

#### Generalized Procedures

Five species of ticks were chosen for these observations: Rhipicephalus sanguineus (Latreille) - as well as being of economic importance in the realm of dog breeders and kennel operators, this species was used successfully by Wood (1911) for rearing H. hookeri, a closely related tick parasitoid; Dermacentor andersoni Stiles has also been used successfully in the mass rearing of H. hookeri by Parker & Butler (1928) and is of economic importance in the livestock industry in the north-western states and is also a known vector of Rocky Mountain Spotted fever; D. variabilis (Say) - was used by other researchers in the mass rearing of H. hookeri (Smith & Cole 1943) and is also a known vector of several diseases in man and domestic livestock; Amblyomma americanum (Linnaeus ) - was selected due to its abundance in many areas throughout the U.S. and its aggressiveness toward man and other mammals, and its relationship to diseases in man and domestic livestock; and A. maculatum - because it has extended it's distributional range a great deal in the past 10 years and poses a great economic threat to the livestock industry of the north-west. It is known to cause paralysis in man and dogs and also creates a feeding lesion on animal hosts which may lead to screwworm Cochliomyia

<u>hominivorax</u> (Coquerel) infestation in areas where the geographical distribution of the two overlap.

The colony ticks used in this study were maintained in the manner described by Patrick & Hair (1975).

The parasitoids used in studies reported herein were maintained by exposing batches of unengorged nymphs (500 to 800 of a particular species and life stage), inside a 1/2 pt 3-3/8" D. paper can with a transparent top of Handi-Wrap<sup>R</sup> held in place by a rubber band; to 80 parasitoids for approximately 48 hrs. Ticks were then placed on sheep hosts and allowed to feed. After dropping from the host they were placed in humidity chambers as previously stated, to allow parasitoids to develop. After 21 days the ticks were examined and those not showing signs of parasitism removed, while those showing evidence of parasitism were maintained as a batch in the humidity chamber to allow adult parasitoids to emerge. As parasitoids from different ticks emerge over a period of 8 to 14 days, they were collected on a daily basis. The parasitoids collected from a batch of ticks on a particular day were kept as a group in the humidity chamber until such time as they were exposed to ticks.

The parasitoid colony material came from <u>I</u>. <u>texanus</u> originating from naturally parasitized <u>H</u>. <u>leporis-palustris</u> recovered in Nowata County in North-eastern Oklahoma during 1978 and which had been maintained for approximately 10 generations on <u>D</u>. <u>variabilis</u>, <u>D</u>. <u>andersoni</u> and <u>R</u>. <u>sanguineus</u> nymphs. In order to eliminate any variation in parasitoid vigor emerging from different tick species all parasitoids used in this study emerged from <u>D</u>. <u>variabilis</u> nymphs.

To show data to meet the objectives of this study, comparisons

were made of the flat larva, replete larva, flat nymphs, and replete nymphs of each of the 5 selected species of ticks to determine species preference. In all comparisons, 100 ticks were exposed to 25 female parasitoids in the manner previously stated for a period of 24 hrs. Flat larvae and nymphs were placed on rabbit host in individual cells immediately after exposure to parasitoids.

Tick restraining cells on rabbits were constructed by cutting 16 oz polyethyene wide mouth bottles, 3/4" below the shoulder and the removal of a 1" diameter plug from the screw cap the plug being replaced by organza cloth to allow air circulation. The cell was attached to the rabbit by first attaching a 2" long piece of tubular orthopedic stockinette 4" diameter to the cell by means of Top-Grip<sup>R</sup> premium grade, general purpose, contact cement, 1" of the stockinette being cemented to the inside of the cell and the other 1" serving as a flange and cemented to the hair of the rabbit. Prior to attaching the cells a small area was clipped free of hair on the back of the rabbit for placement of the cell. Ticks were introduced and retrieved by removing the ventilated screw cap. Replete larvae were placed on hosts 5 days after molting to nymphs was completed. This was necessary due to the phenomenon of latency described by Cooley & Kohl (1934) in which the parasitoids do not develop when ticks are parasitized in the replete larval stage but are dormant until the tick reaches the replete nymph stage. In addition, 100 ticks from the same batches, but not exposed to parasitoids, were handled in the same manner in order to determine degree of tick mortality resulting from parasitism.

The replete larvae and replete nymphs in this study were weighed on a Mettler H51<sup>R</sup> balance accurate to .01 mg and placed inside individual

12 x 75 mm diSPo<sup>R</sup> polyethylene culture tubes fitted with a plastic press fit lid labeled with the tick weight and species, and placed in the humidity chamber until parasitoids emerge or 45 days. After emergence, records of, dates of emergence, number of parasitoids emerging, number of each sex of parasitoids, number of ticks molting, and tick mortality were recorded.

From these records the following determinations were made: (A) The percent successful parasitoid emergence in each tick species, (B) The percent tick mortality due to parasitoids; (C) The average number of parasitoids emerging from each tick species, (D) The sex ratio of parasitoids from each tick species, (E) The average emergence time of parasitoids from each tick species, (F) Any association between tick wt. and number of parasitoids, size of parasitoids and length of emergence time in each tick species.

In order for the results of this study to be more clearly described it is necessary to define certain terms:

<u>Apparent parasitism</u> refers to ticks that are dead and exhibit the external signs of being parasitized. These signs being a swelled appearance of the body and a light to very dark brown color which is not seen in healthy ticks.

<u>Actual parasitism</u> are those ticks in which parasitoids successfully emerge. This term is necessary to separate the effectiveness of the parasitoid from the reproductive potential of the parasitoid.

<u>Drop to emergence</u> (<u>days</u>) refers to the number of days from the time the tick drops from the host following exposure to parasitoids and taking the final blood meal until the day the parasitoid emerge. This time period is not to be confused with the period of time from

exposure of ticks to parasitoids until parasitoid emergence.

<u>Parasitized</u> - <u>no emergence</u> are ticks that have the external signs of being parasitized but there is no emergence of parasitoids. This represents the difference between apparent parasitism and actual parasitism.

#### CHAPTER III

# HOST SUITABILITY OF 5 TICK SPECIES TO IXODIPHAGUS TEXANUS HOWARD

#### Unfed Larvae

Two hundred flat larva (100/cup of each <u>A</u>. <u>americanum</u>, <u>A</u>. <u>maculatum</u>, <u>D</u>. <u>andersoni</u>, <u>D</u>. <u>variabilis</u>, and <u>R</u>. <u>sanguineus</u>), were placed in 1 oz clear plastic portion cups fitted with a paper lid with a removable plug for introducing parasitoids. To one cup of each species, 25 female parasitoids were added, while the remaining cup of each species was not subjected to parasitoids. The 10 containers were then placed in the humidity chamber for 24 hrs at which time the parasitoids were removed and the ticks were placed on rabbit hosts, each group in an individual cell, to receive the blood meal.

After feeding the larvae were weighed, placed in individual culture tubes labeled with the date the tick dropped from the host, tick wt, and species of tick. Ticks were then placed in the humidity chamber until parasitoids emerged or for '45 days.

As illustrated in Table I the actual parasitism of unfed larvae was very poor - being only 1 percent in <u>D</u>. <u>andersoni</u> and 4 percent in <u>D</u>. <u>variabilis</u>. The remaining 3 species had no parasitoids emerge. This abbreviated table illustrates the high percent of mortality in both the treatment and control ticks and may indicate the design of this

## TABLE I

## HOST SUITABILITY OF UNFED LARVAE OF 5 TICK SPECIES TO <u>IXODIPHAGUS TEXANUS</u> HOWARD UNDER LABORATORY <u>CONDITIONS, STILLWATER, OKLAHOMA,</u> SUMMER 1979

	R.s. <sup>a</sup>	D.a. <sup>a</sup>	D.v. <sup>a</sup>	A.m. <sup>a</sup>	A.a. <sup>a</sup>
% actual parasitism	0	1	4	0	0
% molt-control	10	13	0	57	0
% molt-treatment	0	14	0	46	8
% mortality control	90	86	100	43	100
% mortality treatment	100	86	96	54	92
x # parasitoids/tick	0	1	2	0	0
sex ratio female:male	0	1:0	1:1	0	0
drop to emergence (days)	0	32	29	0	0

<sup>a</sup><u>Rhipicephlus sanguineus</u>, <u>Dermacentor andersoni</u>, <u>D. variabilis</u>, <u>Amblyomma maculatum and A. americanum</u>, respectively.

experiment was not in favor of this particular life stage. It was evident that <u>I</u>. <u>texanus</u> would readily oviposit in unfed larvae and that some parasitoids will emerge from engorged larvae. This is opposed to the report of Wood (1911) and Smith & Cole (1943) which state that <u>H</u>. hookeri pay no attention to unfed larvae.

#### Engorged Larvae

Two hundred larvae of <u>A</u>. <u>americanum</u>, <u>A</u>. <u>maculatum</u>, <u>D</u>. <u>variabilis</u>, <u>D</u>. <u>andersoni</u> and <u>R</u>. <u>sanguineus</u> collected within 24 hours following engorgement, were placed in 1 oz portion cups, 100 per cup. To one cup of each species, 25 female parasitoids were introduced, while the remaining cup of each species was not subjected to parasitoids. The parasitoids were removed after 24 hrs and the ticks placed in the humidity chamber until molting was completed. Five days after molting was completed, the ticks were placed on rabbit hosts, each group of 100, in individual cells. After taking the blood meal, the engorged ticks from each group were weighed and placed in individual culture tubes, labeled with the tick weight and species then returned to the humidity chamber for at least 45 days. The dates parasitoids emerged, the number of parasitoids that emerged, and the number of each sex, the number of ticks that molted and tick mortality were recorded during this time.

As illustrated in Table II the apparent parasitism of <u>R</u>. <u>sanguineus</u>, <u>D</u>. <u>variabilis</u> and <u>D</u>. <u>andersoni</u> was 100 percent and indicates that these 3 species were well suited as hosts for <u>I</u>. <u>texanus</u>. Even though actual parasitism in <u>R</u>. <u>sanguineus</u> and <u>D</u>. <u>variabilis</u> dropped slightly 97.9 and 96 percent, respectively, they are still considered highly suitable as

## TABLE II

## HOST SUITABILITY OF ENGORGED LARVAE OF 5 TICK SPECIES TO <u>IXODIPHAGUS TEXANUS</u> HOWARD UNDER LABORATORY CONDITIONS STILLWATER, OKLAHOMA, SUMMER 1979

	R.s. <sup>a</sup>	D.a. <sup>a</sup>	D.v. <sup>a</sup>	A.m. <sup>a</sup>	A.a. <sup>a</sup>
% apparent parasitism	100	100	100	18.8	0
% actual parasitism	97.9	96.0	100	1.2	0
<pre>% parasitized - no emergence</pre>	2.1	4.0	0	17.6	0
% molt-control	98.7	100	100	100	100
% molt-treatment	0	0	0	80	100
replete nymph wt. control (mg)	6.1	13.1	33	20.9	0
replete nymph wt. treatment (mg)	10.2	21.5	51.2	24.3	0
x # parasitoids/tick	17	51	54	13	0
x parasitoid wt. mg	.08	.06	.10	.09	0
sex ratio female:male	3:1	4:1	4:1	6:1	0
drop to emergence (days)	32.3	30.2	33.1	32	0

<sup>a</sup><u>Rhipicephalus</u> <u>sanguineus</u>, <u>Dermacentor</u> <u>andersoni</u>, <u>D</u>. <u>variabilis</u>, <u>Amblyomma maculatum</u> <u>and</u> <u>A</u>. <u>americanum</u>, respectively.

parasitoid host. Cooley (1928) and Smith & Cole (1943) refer to 90 percent parasitism in <u>R</u>. <u>sanguineus</u> and 60.8 percent parasitism in <u>D</u>. <u>variabilis</u> by <u>H</u>. <u>hookeri</u>. Since these researchers did not distinguish between apparent and actual parasitism I will assume they refer to the percent of successful parasitism. It is evident in this table that this life stage of <u>A</u>. <u>maculatum</u> and <u>A</u>. <u>americanum</u> are less suitable as hosts for <u>I</u>. <u>texanus</u> without some parasitoid selection and adaptation to <u>I</u>. <u>texanus</u>.

When comparing the percent molt of treatment and control in <u>R</u>. <u>sanguineus</u>, <u>D</u>. <u>variabilis</u> and <u>D</u>. <u>andersoni</u>, essentially 100 percent of tick mortality can be attributed to parasitism. The 1.3 percent mortality in the <u>R</u>. <u>sanguineus</u> control group does however, express slightly less than 100 percent net mortality due to parasitism in that species.

As illustrated in Table II the replete nymph weight of the treatment and control ticks in all 4 species was affected by <u>I</u>. <u>texanus</u> parasitism, the treatment ticks being heavier than the controls. The significance of this weight increase following parasitism is not known at this time.

With reference to the average number of parasitoids emerging/tick, an average of 17 in <u>R</u>. <u>sanguineus</u> is slightly more than double the average of 8/tick recorded by Wood (1911) by <u>H</u>. <u>hookeri</u>. The average of 51 and 54 parasitoids/tick in <u>D</u>. <u>variabilis</u> and <u>D</u>. <u>andersoni</u>, respectively is far superior to the 20/tick recorded by Smith & Cole (1943) in <u>D</u>. <u>variabilis</u>, or that recorded by Morton (1928) in <u>D</u>. <u>andersoni</u> by <u>H</u>. <u>hookeri</u>. These figures demonstrate the relatively high reproductive potential of <u>I</u>. <u>texanus</u> when exposed to replete larva.

There is a slight difference in the average parasitoid weight among tick species as shown in Table II. It is not known at this time if there is any significance in the difference in parasitoid size. That is, if size is related to fecundity.

The sex ratios of 3:1 for <u>R</u>. <u>sanguineus</u>, 4:1 for <u>D</u>. <u>variabilis</u> and <u>D</u>. <u>andersoni</u>, and 6:1 in <u>A</u>. <u>maculatum</u> female over male as illustrated in Table II by <u>I</u>. <u>texanus</u> and is similar to those ratios found by Smith & Cole (1943) and Wood (1911) by H. hookeri.

The difference in average length in emergence time among tick species did not appear significant as shown by a maximum difference of 3 days between the shortest and longest emergence time between species. Since the average replete nymph weight in treatment ticks of <u>D. andersoni and D. variabilis</u> were 51.2 mg and 21.5 mg, respectively, and the average number of parasitoids produced was almost the same, it would appear that it took longer for the same number of parasitoid larvae to consume more than twice the amount of food mass, thus accounting for the difference in emergence times.

#### Unfed Nymphs

Two hundred newly molted nymphs (5 days after molting was completed) of <u>A</u>. <u>americanum</u>, <u>A</u>. <u>maculatum</u>, <u>D</u>. <u>andersoni</u>, <u>D</u>. <u>variabilis</u>, and <u>R</u>. <u>sanguineus</u> were counted and 100 of each species placed in each of two 1 oz portion cups. Twenty-five female parasitoids were also placed in one cup of each of the 5 tick species while the remaining 100 ticks of each species were not exposed to parasitoids. The parasitoids remained with the ticks for 24 hrs at which time they were removed and the ticks placed on rabbit hosts and allowed to feed. When feeding was

completed, each tick was weighed, placed in an individual culture tube labeled with tick species and weighed and returned to the humidity chamber. Ticks remained in the humidity chamber until parasitoids emerged or for 45 days. Information concerning the dates of parasitoid emergence, the number of parasitoids that emerge, the number of each sex, the number of ticks that molt, and tick mortality were recorded.

As evident from data in Table III representing unfed nymphs the performance of <u>I</u>. <u>texanus</u> in <u>D</u>. <u>andersoni</u> was considerably less than that demonstrated in <u>R</u>. <u>sanguineus</u> and <u>D</u>. <u>variabilis</u> in this tick life stage. The 100 percent apparent parasitism in <u>R</u>. <u>sanguineus</u> and <u>D</u>. <u>variabilis</u> indicated that <u>I</u>. <u>texanus</u> was very effective in these two species, in addition the 97.1 percent actual parasitism for <u>R</u>. <u>sanguineus</u> and <u>91</u> percent actual parasitism in <u>D</u>. <u>variabilis</u> indicated that both species were highly suited for reproduction of these parasitoids. These values were compared to 88.8 percent apparent and 57.5 percent actual parasitism obtained in D. andersoni.

The unfed nymph stage in <u>D</u>. <u>andersoni</u> was less suited for parasitoid reproduction as indicated by 31.2 percent no emergence, as compared to 9 percent for <u>D</u>. <u>variabilis</u> and the low 2.9 percent for <u>R</u>. <u>sanguineus</u>.

The 100 percent molt in control <u>D</u>. <u>variabilis</u> and <u>D</u>. <u>andersoni</u> indicated that all tick mortality in treatment ticks could be attributed to parasitoids. The 11.3 percent molt of treatment ticks in <u>D</u>. <u>andersoni</u> indicated that this species was less suited for <u>I</u>. <u>texanus</u> than <u>R</u>. sanguineus and D. variabilis in this life stage.

With respect to the average replete tick weight shown in Table III, the treatment (= parasitized) ticks were consistantly heavier. These were the same characteristics observed in the replete larva stage as

## TABLE III

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## HOST SUITABILITY OF UNFED NYMPHS OF 5 TICK SPECIES TO IXODIPHAGUS TEXANUS HOWARD UNDER LABORATORY CONDITIONS, STILLWATER, OKLAHOMA, SUMMER 1979

	R.s. <sup>a</sup>	D.a. <sup>a</sup>	D.v. <sup>a</sup>	A.m. <sup>a</sup>	A.a. <sup>a</sup>
% apparent parasitism	100	88.7	100	2.4	0
% actual parasitism	97.1	57.5	91	0	0
<pre>% parasitized - no emergence</pre>	2.9	31.2	9.0	2.4	0
% molt - control	97.4	100	100	100	100
% molt - treatment	0	11.3	0	97.6	100
replete tick wt control (mg)	6.3	30.5	15.1	21.3	9,9
replete tick wt treatment (mg)	8.0	38.8	21.8	20.8	10.0
x # parasitoids/tick	16.2	20.3	40.2	0	0
$\overline{x}$ parasitoid wt. (mg)	0.07	0.13	0.08	0	0
sex ratio	3:1	8:1	6:1	0	0
drop to emergence (days)	34.8	36.6	31.7	0	0

<sup>a</sup><u>Rhipicephalus</u> <u>sanguineus</u>, <u>Dermacentor</u> <u>andersoni</u>, <u>D</u>. <u>variabilis</u>, <u>Amblyomma</u> <u>maculatum</u> and <u>A</u>. <u>americanum</u>, respectively.

represented in Table II.

The  $\overline{x}$  number of parasitoids (40.2) emerging/tick from <u>D</u>. variabilis was desirable from the standpoint of mass rearing of <u>I</u>. <u>texanus</u>. This figure represented twice the reproductive potential of <u>H</u>. <u>hookeri</u> reported by Smith & Cole (1943). While the reproductive figure of 16.2 remained almost constant with the  $\overline{x}$  of 17 in Table II representing engorged larvae for <u>R</u>. <u>sanguineus</u>, there was a drastic decline in # parasitoids/tick in <u>D</u>. <u>andersoni</u>, indicating that the unfed nymphal stage of <u>D</u>. <u>andersoni</u> was probably less suited for <u>I</u>. <u>texanus</u> development.

The average parasitoid weight data in Table III representing unfed nymphs was very near that found in Table II representing replete larvae. The parasitoids emerging from <u>D</u>. <u>andersoni</u> (.13 mg) were much larger than those emerging from <u>R</u>. <u>sanguineus</u> (.07 mg) and <u>D</u>. <u>variabilis</u> (.08 mg).

The sex ratio in Table III was about the same as that in Table II for <u>R</u>. <u>sanguineus</u> (3:1). However, there was a considerably higher ratio of female to male in <u>D</u>. <u>andersoni</u> (8:1) and <u>D</u>. <u>variabilis</u> (6:1). This quality may be very important with respect to reproductive rates in mass rearing.

The emergence time from drop to emergence in unfed nymphs was slightly longer than that recorded in Table II representing replete larvae. Parasitoids emerging from <u>D</u>. <u>variabilis</u>, in 31.7 days continued to have the shortest emergence time. This was 13 days shorter than that found by Cooley (1928, 1930) at the same optimum temperature of 22°C. Smith & Cole (1943) recorded emergence times of from 20-30 days at 24°C, which indicated that temperature may have a great influence on emergence time.

#### Engorged Nymphs

Two hundred replete nymphs of <u>A</u>. <u>americanum</u>, <u>A</u>. <u>maculatum</u>, <u>D</u>. <u>variabilis</u> and <u>R</u>. <u>sanguineus</u> collected within 24 hrs after engorgement were placed in 1 oz portion cups, 100 in each cup. Twenty-five female parasitoids were added to one cup of each of the 5 species of ticks while no parasitoids were added to the remaining 5 cups. Ticks were exposed to parasitoids for a period of 24 hrs, at which time they were removed and ticks weighed, placed in individual culture tubes labeled with tick weight and species, and returned to the humidity chamber for 45 days to allow time for parasitoids to emerge. During this time the dates parasitoids emerged, the number of parasitoids that emerged, the number of each sex, the number that molted and tick mortality were recorded.

Apparent parasitism near 100 percent (Table IV) was very good in <u>R. sanguineus, D. andersoni and D. variabilis</u>. Although <u>A. americanum</u> was unsuitable in this life stage, exposed <u>A. maculatum</u> showed 64 percent apparent parasitism, which represented an acceptable degree of host suitability in this life stage. The 38 percent actual parasitism in <u>A. maculatum</u> was adequate to justify further genera adaptation studies with this species and <u>A. americanum</u>.

There was a pronounced decline in actual parasitism in <u>R</u>. <u>sanguineus</u>, <u>D</u>. <u>variabilis</u> and <u>D</u>. <u>andersoni</u> in this life stage not seen with the replete larval and unfed nymphal stages.

The percent apparent parasitized but no emergence of 30, 15, 12, and 27 respectively for <u>R</u>. <u>sanguineus</u>, <u>D</u>. <u>andersoni</u>, <u>D</u>. <u>variabilis</u> and <u>A</u>. <u>maculatum</u> were relatively high and therefore made this life stage

# TABLE IV

## HOST SUITABILITY OF ENGORGED NYMPHS OF 5 TICK SPECIES TO IXODIPHAGUS TEXANUS HOWARD UNDER LABORATORY CONDITIONS, STILLWATER, OKLAHOMA, SUMMER 1979

	R.s. <sup>a</sup>	D.a <sup>a</sup>	D.v. <sup>a</sup>	A.m. <sup>a</sup>	A.a. <sup>a</sup>
% apparent parasitism	100	99	100	64	0
% actual parasitism	70	84	88	38	0
% parasitized - no emergence	30	15	12	27	0
% molt - control	83	100	100	99	100
% molt - treatment	0	1	0	36	100
replete tick wt. – control (mg)	5.3	25.9	10.4	15.3	0
replete tick wt treatment (mg)	5.3	26.2	10.0	15.5	0
x # parasitoids/tick	16	41	25	16	0
$\overline{x}$ parasitoid wt. (mg)	.03	.06	.05	.08	0
sex ratio female:male	2:1	5:1	3:1	4:1	0
drop to emergence (days)	39.7	38.3	37.2	39.0	0

<sup>a</sup><u>Rhipicephalus</u> <u>sanguineus</u>, <u>Dermacentor</u> <u>andersoni</u>, <u>D</u>. <u>variabilis</u>, <u>Amblyomma maculatum</u> and <u>A</u>. <u>americanum</u>, respectively. seem less desirable for maximum mass production. The exception to this being <u>A</u>. <u>maculatum</u> in which this stage represented the only developmental stage of this species to show significant parasitoid production.

As illustrated in Table IV virtually 100 percent of the mortality in <u>D</u>. <u>andersoni</u>, <u>D</u>. <u>variabilis</u>, and <u>A</u>. <u>maculatum</u> can be attributed to parasitoids while 17 percent of the control <u>R</u>. <u>sanguineus</u> did not molt indicating possible error in handling. The 36 percent molt in <u>A</u>. <u>maculatum</u> is not surprising since this is the only stage that indicates a satisfactory rate of parasitism in this species.

Data related to average replete tick weight was of little significance in this table since treatment ticks were exposed to parasitoids after engorgement. These weights were taken to insure tick uniformity throughout the study.

Average number of parasitoids emerging/tick from <u>R</u>. <u>sanguineus</u> (Table IV) was about the same as that from replete larvae and unfed nymphs. The 4l parasitoids/tick in <u>D</u>. <u>andersoni</u> reflect a high degree of susceptibility of replete nymphs of this species. <u>D</u>. <u>variabilis</u> with 25 parasitoids/tick represented a slight decline in parasitoid production relative to the replete larva and unfed nymph stages. <u>A</u>. <u>maculatum</u> produced 16 parasitoids/tick and represented an effective rate of production in that species whereas other developmental stages were unsatisfactory.

The average parasitoid weights from <u>R</u>. <u>sanguineus</u>, <u>D</u>. <u>andersoni</u>, and <u>D</u>. <u>variabilis</u> were substantially less than those recorded in Table II and III. It was not known if the parasitoid size had any relationship to fecundity. Parasitoids with an average weight of .08 mg

emerging from <u>A</u>. <u>maculatum</u> reflect a good conversion factor of the body content of this tick species by <u>I</u>. <u>texanus</u> when compared to .06 mg for <u>D</u>. <u>andersoni</u> and .05 mg for <u>D</u>. <u>variabilis</u>.

The sex ratios in Table IV were not significantly different than those in Tables II and III. The ratio of 4:1 for <u>A</u>. <u>maculatum</u> again reflected a possible suitability factor for that species with this stage of development.

The emergence times of 39.7, 38.3, 37.2 and 39.0 days for <u>R</u>. <u>sanguineus</u>, <u>D</u>. <u>andersoni</u>, <u>D</u>. <u>variabilis</u> and <u>A</u>. <u>maculatum</u>, respectively were considerably longer than those recorded in the replete larval and unfed nymphal stages. This longer emergence time strongly indicated that <u>I</u>. <u>texanus</u> eggs hatch immediately after nymphs began the feeding process. This was made more evident when the 5 days required for <u>D</u>. <u>andersoni</u> to feed was added to the average emergence of 33.1 days for engorged larvae. This gave a sum of 38.1 days which compared to 38.3 in Table IV for the engorged nymph stage.

#### CHAPTER IV

#### SUMMARY

The objective of this study was to determine the laboratory biology of <u>I</u>. <u>texanus</u> in order to define its effectiveness against different tick species and life stages. From these data the best suited tick species and life stage for colony rearing, and mass rearing for possible future release as biological control agents might be made.

Findings from this study indicate that all immature stages of <u>R</u>. <u>sanguineus</u>, <u>D</u>. <u>andersoni</u> and <u>D</u>. <u>variabilis</u> were suitable host for <u>I</u>. <u>texanus</u> under laboratory conditions. Findings also show that <u>A</u>. <u>maculatum</u> with its relatively low percent parasitism may offer an avenue for genera adaptation by the parasitoids which would eventually be effective against <u>Amblyomma americanum</u> and other <u>Amblyomma</u> species.

Results also showed that engorged <u>D</u>. <u>variabilis</u> larvae exhibited the best overall host qualities for colony rearing as well as mass rearing of <u>I</u>. <u>texanus</u>. These qualities were demonstrated by 96 percent actual parasitism and a  $\overline{x}$  of 50.7 parasitoids emerging/tick. <u>D</u>. <u>variabilis</u> also possesses the advantage of being easily reared and maintained in colony. Further, only one stage had to be fed before the host was ready for exposure to parasitoids.

As illustrated in this study by the difference in emergence times of those ticks exposed prior to nymphal engorgement and those exposed

after nymphal engorgement, <u>I</u>. <u>texanus</u> eggs hatched almost immediately after the nymph began the feeding process. This information may prove very valuable in storage of these parasitoids for shipment or for mass release.

Although the generalized life history and biology of <u>I</u>. <u>texanus</u> and <u>H</u>. <u>hookeri</u> appear to be very similar, this study depicts three positive attributes of <u>I</u>. <u>texanus</u> not present in <u>H</u>. <u>hookeri</u>: (1) a much greater reproductive potential; (2) a greater variety of tick host species and life stages affected; (3) and the trait of <u>I</u>. <u>texanus</u> to parasitize the host in vitro and give a high percent of successful parasitism.

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#### Thesis: HOST SUITABILITY AND LABORATORY BIOLOGY OF <u>IXODIPHAGUS</u> TEXANUS HOWARD

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