THE BIOLOGY AND VECTOR CAPABILITY OF *TRIATOMA SANGUISUGA TEXANA* USINGER AND *TRIATOMA GERSTAECKERI* (STAL) COMPARED WITH *RHODNIUS PROLIXUS* (STAL) (HEMIPTERA: TRIATOMINAE)

By

WARREN FLOYD PIPPIN

Bachelor of Science
Colorado State University
Fort Collins, Colorado
1941

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1958

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Thesis approved:

D. E. Hoxell
Thesis Adviser

R. R. Walton

Winston B. Graves

N. Durham
Dean of the Graduate College

PREFACE

The presence of Trypanosoma cruzi, the causative agent of American trypanosomiasis, has been confirmed and well documented in the United States. The biology and vector effectiveness of many species of Triatoma that transmit this organism are not well known. In particular, studies that directly compare South American and United States species have been limited. It was decided that a comparative study of two Texas species with that of a highly effective South American vector would be desirable. Data were taken during the period November, 1964 through November, 1967.

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CHAPTER I

INTRODUCTION

A parasitic hemoflagellate, Trypanosoma cruzi Chagas, is the causative agent of Chagas' disease or American trypanosomiasis. This parasite has been recovered in nature from several species of mammals found in the south and southwestern United States. In addition, this same organism occurs naturally in many species of indigenous blood sucking insects belonging to the order Hemiptera, subfamily Triatominae. In 1909, Carlos Chagas, working in Brazil, somewhat clarified the epidemiology of the disease by tracing the life cycle of the trypanosome from mammal reservoir to man through infected triatome bugs.

Despite this natural reservoir-vector relationship, and the relative prevalence of Chagas' disease throughout South and Central America and Mexico, no infections were reported in the United States until 1955. In that year, two indigenous cases were recorded from south Texas. Subsequent serological studies in Texas have shown complement fixing antibodies in the blood of nine of 500 humans tested. It is not completely known why this disease is not more prevalent in humans in the southwestern part of the United States. There is considerable evidence that humans frequently contact blood sucking triatomid bugs that harbor the protozoan Trypanosoma cruzi. One of several reasons suggested for the comparatively low human incidence of Chagas' disease in the United States is differences in the habits of the insect vectors.

It is essential to study and document the biology of suspected or known vectors in order to understand the epidemiology of a vector-borne disease. Triatoma sanguisuga texana Usinger and Triatoma gerstaeckeri (Stal) are two of several species of triatomid bugs found in Texas. Both hemipterans occupy the dwellings of woodrats and other mammals that may be a reservoir of Trypanosoma cruzi. Both species have been found naturally infected with Trypanosoma cruzi, and there are recorded instances where each was a pest in human habitations. The biology of neither of these two species of bugs is well known. In particular, data comparing the vector capabilities of these two species of bugs are unavailable. For these reasons, it was decided to compare the biology and vector capabilities of Triatoma sanguisuga texana and Triatoma gerstaeckeri with those of Rhodnius prolixus (Stal), an effective and well studied vector of Chagas' disease in South America.

The objectives of this study, instituted in late 1964, were to (a) study the biology of two species of triatomid bugs found in Texas; (b) compare the biology of the two Texas species with that of a South American species; and (c) compare the ability of the three species of bugs to transmit both a Texan and Brazilian strain of Trypanosoma cruzi.

CHAPTER II

REVIEW OF THE LITERATURE

The literature pertaining to American trypanosomiasis is voluminous and no attempt has been made to review all aspects of the subject. Only those areas that may be of general interest or that relate to the problem delineated will be dealt with here.

Historical

Carlos Chagas (1909) reported a new disease of man in the State of Minas Geraes, Brazil. The causative agent was a protozoan belonging to a group known as the hemoflagellates. Chagas (1921) wrote that the discovery of American trypanosomiasis was made under conditions which differ from the usual course of events in pathology. In this case, knowledge of the parasite preceded that of the disease caused by it. Data pertaining to the biology of the transmitting insect suggested investigations that resulted in the finding of a new nosographic entity.

Chagas was in Minas Geraes on an antimalarial campaign for the Oswaldo Cruz Institute when he noted the presence of haemophagous insects in most of the houses in that area. He examined specimens of these insects and found flagellates in their digestive tract. Several of these insects were sent to Cruz in Rio de Janeiro where he fed them on monkeys of the genus Callithrix. Fifteen or

twenty days after contact with the insect, one of the monkeys showed signs of a disease in which an acute keratitis was prominent. Examination of the blood of this animal demonstrated the presence of a trypanosome. Later experiments demonstrated this organism was transmitted by the species of the bug found in human residences in Minas Geraes.

New investigations were conducted in Minas Geraes to find the normal host of the parasite (Chagas 1921). First work on the subject resulted in finding the parasite in the peripheral blood of a domestic cat. Subsequent examination of the blood of a three-month old child revealed a large number of trypanosomes of a morphology identical to those transmitted by infected insects to monkeys.

There has been some confusion in the literature concerning the proper nomenclature of the parasite causing American trypanosomiasis. Hoare (1934) reported the parasite was originally named Trypanosoma cruzi Chagas, 1909. Having discovered what he believed to be stages of schizogony of this flagellate in the lungs of the mammalian host, Chagas renamed it Schizotrypanum cruzi Chagas. It was later demonstrated that these stages belonged to a different organism. Chagas then reverted to the original name, T. cruzi, however, the name S. cruzi is still found occasionally in South American literature.

Since the pioneer work of Chagas, the disease has been considered a zoonosis. Deane (1963) reported that for sometime the ailment was thought to be a domestic zoonosis, i.e., a disease attacking only man and domestic mammals, transmitted by a triatomid bug, Panstrongylus megistus (Burm). This bug was then thought to be purely domestic. Deane further reports that Chagas

in 1912, found specimens of P. geniculatus (Latreille) from burrows of armadillos harboured flagellates very similar to those from P. megistus. Chagas examined the blood of several armadillos and discovered similar trypanosomes which were infective for guinea pigs. Chagas decided he had found a wild reservoir and wild vector of T. cruzi. Since the mammalian host probably evolved before man and the bug was known to have wide distribution in South and Central America, Chagas assumed that the disease was primitively sylvan, later adapted to human dwellings and also widespread on the Continent.

Distribution and Incidence of the Disease

For many years Minas Geraes, Brazil appeared to be the only area where human cases were common. Interest in the disease, coupled with improved complement fixation tests and xenodiagnosis have demonstrated that T. cruzi is widespread in humans and other mammals in the Western Hemisphere. The World Health Organization (1960) estimated that 7,000,000 people were infected and 30,000,000 exposed to the disease. Most of the acute and chronic infections are found in South America. The disease is known to range from Latitude 41° South to 38° North (Wood and Wood 1941).

Woody and Woody (1961) state that detection of the parasite has followed a stereotype pattern in each country where it has been isolated. First, the animal reservoirs and their associated transmitting insects (the reduviid bugs) were discovered. A few infections in man were then recognized and reported. The finding of disease in humans then stimulated surveys using laboratory diagnostic methods. When these unearth examples of unrecognized infection, clinical

interest was excited and increased numbers of cases were discovered in humans.

Woody and Woody (1955) reported the first indigenous case of American trypanosomiasis in the United States in Corpus Christi, Texas. A second case was reported by Greer (1955) near Houston, Texas. Serological tests indicate that inapparent American trypanosomiasis might occur more frequently in the United States than previously thought. In 1942, complement fixation tests were performed on 1,909 serum samples from persons in different parts of Texas; only one was postive (Davis, Sullivan, and deShazo 1946). A serological survey of 500 patients admitted to Children's Hospital in Corpus Christi, Texas revealed nine positive cases, a rate of 1.8% (Woody and Woody 1961). Farrar et al. (1963) demonstrated that among 28 patients in the cardiac clinic at Grady Memorial Hospital in Atlanta, Georgia with a diagnosis of "diffuse" myocardial disease, two (7.1%) had positive reactions to T. cruzi. Of 474 unselected sera obtained from the serology laboratory of the same hospital, only two (0.4%) were positive. A similar number of positive reactions was found in a series of 449 unselected sera obtained from various rural counties in northern Georgia. Sera from 117 persons who had been bitten by triatome bugs in Texas were tested for complement fixation with T. cruzi antigen; three (2.5%) were positive (Woody, Hernandez, and Suchow 1965). Lathrop and Ominsky (1965) reported one positive serum in 108 tested from an area near San Antonio, Texas.

Excellent descriptions of the disease in man are given by Yorke (1937), Woody and Woody (1961), and Winslow and Chaffee (1965).

Mammalian Reservoirs

The problem of animal reservoirs of T. cruzi has interested many workers since Chagas isolated the organism from a domestic cat and armadillos. As a result of that interest, Deane (1963) reported that trypanosomes similar to T. cruzi have been found in over 100 species or sub-species of mammals; 42 of these, eight domestic and 34 wild, in Brazil alone. Wood and Wood (1941) and Usinger (1944) listed known reservoir animals.

Wood (1934) reported the first known naturally infected mammal in the United States; Neotoma fuscipes macrotis from California. Since then the organism has been recovered from wild mammals in Texas, Arizona, Maryland, Georgia, Florida, Louisiana, and Alabama.

Wild mammals found naturally infected with T. cruzi in Texas include the nine-banded armadillo, Dasypus novencinctus texana; house mouse, Mus musculus; opossum, Didelphis virginiana; and the wood rat, Neotoma micropus micropus (Packchanian 1942). Lathrop (1964) isolated the organism from the ring tailed cat, Bassariscus astutus, in an area near San Antonio, Texas.

Vectors

The first reported vector of T. cruzi was P. megistus in Brazil (Chagas 1909). Reviews by Wood and Wood (1941) and Usinger (1944) listed the species groups that were vectors in South and Central America, and Mexico.

Kofoid and McCulloch (1916) were the first to report a trypanosome from a triatome bug in the United States. They first isolated the organism from

Triatoma protracta collected in California. The organism was believed to be a new species and was named T. triatomae. Kofoid and Donat (1933) later reported that T. triatomae was in fact T. cruzi. In addition to California, naturally infected bugs have been reported from Arizona, New Mexico, Texas, Utah, Louisiana, and Alabama (Olsen 1964). Ryckman (1965) stated that the species groups of Triatominae found infected in the United States were Triatoma protracta, T. recurva (=longipes), T. sanguisuga, T. rubida, T. lecticularius, T. gerstaeckeri, and T. neotomae.

In Texas, Packchanian (1939) was the first to report the natural infection in a triatome bug. He isolated the organism from specimens of T. gerstaeckeri, collected near the town of Three Rivers. The following year Packchanian (1940) also reported T. heidemanni (=T. lecticularius) as naturally infected. Wood (1941) added T. protracta woodi and T. rubida to the list. Davis et al. (1943), in a survey of the potential problem of American trypanosomiasis in Texas, found T. sanguisuga and T. ambigua (=T. sanguisuga ambigua of Usinger 1949) naturally infected. The latter record is unusual in that ambigua is a Florida subspecies of sanguisuga. Pippin, Law, and Gaylor (1968) were the first to report the natural infection of T. sanguisuga texana and T. sanguisuga indictiva in Texas.

Infection Rate in Triatome Bugs

The infection rate in bugs found naturally infected varies considerably with the geographical location. Olsen (1964) stated that the infection rate for over 3,500 western and southwestern Triatoma examined by various investigators is about 25%. Yeager (1960) said that the overall rate of infected bugs collected in

this country had been approximately 20%, which is similar to the rate observed in South America.

The overall infection rate in Texas has been found to be higher than in any other state where T. cruzi has been reported; probably because it has been more intensively studied. Ninety-two percent of 100 live T. gerstaeckeri were naturally infected with trypanosomes (Packchanian 1939). Wood (1941a) found three (5.5%) of 54 T. gerstaeckeri infected but none of 10 T. sanguisuga. Wood (1941b) reports two infected T. gerstaeckeri nymphs from a new locality in Texas. Davis et al. (1943) examined nine T. sanguisuga from two counties in Texas and found four (44%) infected. In the most extensive survey in Texas, Sullivan et al. (1949) collected 1,631 specimens of Triatominae in 40 counties; of these, 859 were examined for T. cruzi and 226 (33.3%) were infected. Ninety T. sanguisuga were examined and 23 (25.5%) were infected. Four hundred and fifty T. gerstaeckeri were examined and 135 (29.9%) were infected. The infection rate in adults was higher than in nymphs; species and number of adults were not mentioned. Elkins (1954) collected three species of Triatoma in north central Texas. Sixteen T. gerstaeckeri were examined and none found infected but of 167 T. sanguisuga examined, four (2.4%) were infected. Eads, Trevino, and Campos (1963), in a survey of South Texas wood rat dens, reported 50 (22%) of 226 T. sanguisuga were infected. In the same survey they found 84 (63%) of 133 T. gerstaeckeri were infected.

Bionomics of Triatome Bugs

General.--The bionomics of South American species of the Triatominae

have been well documented, especially Rhodnius prolixus (Uribe 1927, Buxton 1930, Gilliard 1935, Gomez-Nunez 1963, 1964). The publications of Wigglesworth (1931-1936) on the physiology of R. prolixus are classics. The bionomics of North American species are less well known. Usinger (1944) reviewed the known aspects of the biology of several North American species. Ryckman (1962) published a comprehensive study of the T. protracta complex in North America. Wood, in almost all of his many publications referred to limited observations on the life history of several North American species.

T. sanguisuga.--Grundemann (1947) working in an area near Manhattan, Kansas, reported the first extensive observations on the life history of T. sanguisuga. On the basis of laboratory rearing and by measurement of the head capsule of nymphs he concluded there were eight nymphal instars in this species. The life cycle was deduced to be approximately three years under natural conditions in Kansas. Seventy-five percent of the nymphs reportedly molted after a single engorgement. He reported that humidity did not affect the development of eggs. Eggs were not deposited until feeding and fertilization had taken place. One fertilization was apparently sufficient to fertilize all eggs laid by a female in her lifetime. Grundemann concluded that newly-molted adults migrate from wood rat nests for fertilization. Olsen (1964) in Alabama found five nymphal instars in T. sanguisuga. Under laboratory conditions he found that females deposited an average of 711 eggs in their lifetime. A cyclic variation with time was observed in egg laying. Egg development and hatching was inhibited by low humidity. Nine adult females reared from fifth instar nymphs collected in the field lived an average of 456 days and six males an average of 526 days. Olsen also

reported that adults reared from nymphs collected in nature lived longer than those reared from eggs laid in the laboratory. Hays (1965) in a study in Alabama presented the same data as Olsen on longevity and egg laying. Hays also reported considerable difficulty in rearing T. sanguisuga from eggs laid in the laboratory. He stated these insects could not be reared past the third nymphal instar in clean cages. Adults were reared only in cages that contained or had contained field collected insects.

T. gerstaeckeri.--Observations pertaining to the life history of T. gerstaeckeri have been limited. Usinger (1944) reported that eggs hatched in 17 days, without mentioning temperature or humidity. He said eggs were large, oval, and white, and laid without adhesive. A single pair produced 64 eggs in one week. Wood (1941) mentioned that one laboratory raised female lived 119 days on five feedings, a male 53 days on two feedings, and another male 119 days on five feedings. One female collected in the field lived 40 days in the laboratory on one feeding. Thurman (1945) reported that in a seven month period, females in a colony laid an average of 245 eggs per female. One female laid 502 eggs in 152 days.

Feeding Habits.--The Triatominae habitually live on the blood of vertebrate animals (Usinger 1944). However, observations made many times indicate that various species of Triatominae will occasionally feed on their own or other species and ingest blood or haemolymph (Brumpt 1914, Torres 1915, Diaz 1936, Wood 1941, Ryckman 1951, Phillips 1960b, Olsen 1964, Hays 1965, Marinkelle 1965). The nomenclature applied to this feeding process appears to be open to question. Brumpt (1914) called it cannibalism. Ryckman (1951) proposed the

term "kleptohemodipnonism" to designate the "act of stealing blood." Phillips (1960b) proposed that Ryckman's term should be retained, not to replace "cannibalism" but in addition to it, although in a less unwieldy form such as "haematoklepty." Hays (1965), unable to demonstrate the ingestion of blood by various instars of T. sanguisuga from donor bugs, proposed the term intraspecific parasitism to designate the uptake of haemolymph and possibly other fluids.

The feeding of Triatominae on their own kind in maintaining T. cruzi in nature is in question. Results of transmission attempts in the laboratory by this method have been variable. Torres (1915) was unable to transmit T. cruzi to nymphs of E. sordida from a donor bug. Diaz (1934) reported that Chagas experimentally transferred T. cruzi between individuals of P. megistus by cannibalism. Diaz (1936) found that 13 of 44 nymphs of P. megistus become infected after feeding on infected bugs. Phillips (1960b) reported inter-vector transmission by cannibalism to all of 12 first instar R. prolixus from donors of the same species, to eight of 10 first instar T. infestans from donors of the same species, and to two of five first instar T. infestans from R. prolixus donors. Hays (1965) was unable to achieve transmission between various instars of T. sanguisuga that ingested only haemolymph. Marinkelle (1965) demonstrated inter-vector transmission in 23 of 1,000 R. prolixus that fed on infected donor bugs of the same species.

The possibility of triatome bugs ingesting their own or other bug feces has been the subject of considerable controversy. Chagas (1909) quoted Lutz as initially suggesting that transmission of T. cruzi may take place naturally be-

tween individual bugs by means of coprophagy. Brumpt (1914) was apparently the first to report this practice among individuals of R. prolixus. He suggested this was a means of maintaining intestinal fauna common to all Triatominae. Diaz (1934, 1936) was unable to infect first instar nymphs of P. megistus by exposure to bug feces. Phillips (1960b) reported that five of about 400 R. prolixus nymphs practiced coprophagy under conditions of extreme starvation. Marinkelle (1965) exposed 100 nymphs of R. prolixus to eosin stained feces and found that two fed. He also stated that the mechanism of transmission probably includes ingestion of fecal deposits.

Size of Blood Meal.--The amount of blood consumed by various nymphal instars and adults of triatomid bugs is important in the maintenance of T. cruzi in nature. It would seem logical that the larger the amount of blood ingested, the greater the chance of the bug becoming infected. This would be particularly true when small numbers of organisms were circulating in the peripheral blood. The literature is not extensive on this subject. Wigglesworth studied the amount of blood taken at successive meals by R. prolixus and the facts were included, by permission, in Buxton (1930). The blood ingested at one meal varied from approximately 6 mg in first instar nymphs to 284 mg in fifth instar nymphs. Adults averaged 172 mg. Hays (1965) reported that adult female T. sanguisuga consumed an average of 61 mg of blood per meal and males 43 mg. He also stated that no information on the amount of food consumed by North American Triatominae was found in the literature. Thurman (1945a) found that blood ingested averaged from 3.9 mg in first nymphal instars of T. gerstaeckeri to 192.3 mg in the fifth instar. Wood (1959a) reported the amount of blood ingested by nymphs

and adults of T. protracta and T. rubida.

Host Relationships.--The Triatominae appear to have definite host preferences, although they may occasionally feed upon a wide variety of vertebrate hosts. Some species such as R. prolixus have become so adapted to human habitation that they might be considered as domesticated (Usinger 1944).

The most widely distributed and largest host complex in North America appears to be wood rats of the genus Neotoma. However, other animals may be primary hosts in certain localities. Packchanian (1939) reported that in Florida, T. sanguisuga ambigua colonized in the "boots" of palmetto trees and fed on tree toads of the genus, Hydra. Elkins (1951) collected several species of Triatominae in north central Texas, but found no wood rats. He was unable to find a preferred host in this area. Olsen (1964) suggested that the opossum and racoon have replaced wood rats as the preferred host of T. sanguisuga in the southeastern states.

Geographical Distribution.--Most of the 80-odd species of Triatominae occur in the Western Hemishphere, but there is a monotypic genus in India, and one small group of six species in southeast Asia (Usinger, Wygodzinsky, Ryckman 1966).

The reported geographical distribution of any species of insect is probably directly proportional to the time and effort spent looking for it. R. prolixus is considered to range from northern South America, through Central America into southern Mexico (Usinger 1944). T. gerstaeckeri is said to occur over much of the State of Texas (Eads 1963). This species is also found in northern Mexico (Usinger 1944) and southeastern New Mexico (Wood 1961a). The distribution of T. sanguisuga texana is apparently limited to southwest Texas (Usinger 1944).

Dispersal.--In general the dispersal of insects is accomplished by flying, walking, swimming, drifting with air or water currents, or by clinging to objects that move or are moved from place to place. Since most species of Triatominae are capable of sustained flight, it would appear that this method of dispersal would be important. However, Gomez-Nunez (1964) stated that R. prolixus is reluctant to fly and depends on other methods of transport for its dispersal. He suggested that some of these methods are: as ectoparasites of birds, on clothing, paper, cardboard containers, and construction materials.

Observations on the flight habits of North American species of Triatominae have been limited. Sjogren and Ryckman (1966) reviewed the literature and reported on the nocturnal flight habits of T. protracta protracta in Southern California. They propose that when the interior of the wood rat lodge becomes sufficiently warm, during periods of high temperature, the hungry adult bugs are stimulated to leave the nest in search of food. They also found that 63% of the bugs collected in their traps were taken within 30 minutes after zero foot-candles of light intensity.

In Vitro Feeding

The ability of haemophagous arthropods to feed through a variety of membranes is well documented (Tarshis 1956). Nicolle (1941) and Nicolle and Mathis (1941) showed that it was possible to feed T. infestans on several different diets through a Durex rubber film. A simple apparatus for the artificial feeding of R. prolixus was described by Harrington (1960). He obtained good results by feeding through thin Durex rubber. Gomez-Nunez (1963) stated that

mass feeding of R. prolixus through rubber membranes was attempted without success. The unsuccessful feeding of T. infestans and R. prolixus through a rubber membrane has been reported by Shimamune et al. (1965). They found that fresh rat skin or guttapercha membranes gave the best results. Information was unavailable in the literature concerning the ability of T. sanguisuga or T. gerstaeckeri to feed through membranes.

Transmission and Vector Efficiency

Transmission.--Hoare (1934) in his review of the transmission of American trypanosomiasis stated there has been a long standing controversy between the followers of Chagas, who contended that T. cruzi is transmitted through the bite of an infected bug, and those of Brumpt who maintained that transmission is by fecal contamination. Hoare further said that the work of Diaz largely confirms and amplifies Brumpt's observations, but Diaz was unable to substantiate most of Chagas' statements. The mass of evidence accumulated in the literature to the present indicates that T. cruzi is not transmitted by the bite of infected bugs.

The possibility of transmission by other methods has been suggested. Chandler (1949) commented that mammals may become infected by eating other infected mammals. It has been demonstrated experimentally, in mice, that trypanosomes could be passed from the mother to the offspring by the mammae (Craig and Faust 1951). The possibility of mammal to mammal transfer by infective urine has been suggested (McKeever et al. 1958). Ryckman (1965) stated the ingestion of kissing bugs infected with T. cruzi is the principal means by which

wood rats are infected, and that fecal contamination per se is of secondary importance.

Some of the requirements for effective transmission are that the vector is infective to the host and that the host acquires and maintains a parasitemia high enough to infect other vectors over a period of time. Generally, most zoonoses produce characteristically mild or sub-clinical infections in their wild host. Pizzi (1961) observed age differences in susceptibility to T. cruzi infections in rats. He concluded this was due primarily to an increased ability to mobilize phagocytes of increased efficiency by older rats. Olsen (1964) states that it was almost impossible to produce a detectable parasitemia in rats older than 20-25 days. He also found that the parasitemia peaked at about 21 days and almost completely disappeared after 33 days. With the passing of time there is a progressive diminution in the possibility of infected wood rats to infect Triatominae with T. cruzi (Ryckman 1965).

Vector Efficiency.--Wheeler and Douglas (1941) defined vector efficiency as "an experimentally determined numerical value which represents the average number of transmissions effected by a given individual of a particular species of vector under precisely defined and controlled conditions." Three factors contribute to obtaining this numerical index: (1) infection potential, the percentage of a vector species that become infected; (2) vector potential, the percentage of infected vectors that become infective and (3) transmission potential, the mean number of transmissions by a group of infected individuals (Phillips 1960a).

The ability of T. cruzi to develop into an infective form in its vector is, of course, important in the epidemiology of the disease. Phillips and Bertram

(1967) reviewed the literature and calculated the infection rates on several species of Triatominae. They found that 14 or more days after ingesting trypanosomes, infection rates for metacyclic or crithidia forms in bug feces were between 78% and 92% for nymphal R. prolixus. The older nymphs were least susceptible. Adults of R. prolixus were less susceptible (64-98% positive) than three other species tested.

There is ample evidence that the susceptibility of triatome bugs to infection with T. cruzi varies with the species of bug, strain of the organism, and geographical location (Diaz 1940a, b, Zeledon and Vieto 1957, Phillips 1960a, Ryckman 1965, Little et al. 1966).

CHAPTER III

MATERIALS AND METHODS

Laboratory Procedures

Rearing Bugs.--Stock cultures of all three species of Triatoma were maintained in the laboratory and treated alike. Each species were kept in separate circular bell jars. Jars of two different sizes were used; one 11-3/4 inches and the other 8 inches by 8 inches. A circular piece of paper toweling or blotting paper was placed in the bottom of each jar to absorb moisture. A two-inch thick piece of styrofoam was cut to fit each jar. Four grooves 2-1/2 inches long and 1-1/2 inches wide were cut in the underside of the styrofoam disc. Access to each groove, from the upper surface of the disc, was provided by a hole one-inch square. A rectangular piece of styrofoam 1 inch by 2 inches was glued to the surface of the disc to provide a handle (Figure 1). The above procedure allowed the bugs to hide in the grooves on the underside of the styrofoam disc and emerge through the holes to feed. To prevent escape, a two-inch band of petrolatum was smeared around the inside of each jar near the top. A piece of cloth was then placed over the jar opening and, in the case of the larger jars, secured with a large rubber band. A stainless steel animal jar top was placed over the cloth on the smaller jars. Hundreds of specimens of all stages could be reared in the manner described without difficulty.

Individual and paired bugs, for experimental purposes, were kept in six and 10 dram plastic vials with snap-on plastic caps. A circular hole 3/4 to 1 inch in diameter was cut in the top of each cap. A piece of circular nylon screening was then glued to the inner surface of the cap. Thirty-two mesh screening was used to confine first instar nymphs and 20 mesh screening for all other stages. A strip of styrofoam 3/16 inches thick and 1/2 inch wide was glued inside each vial to provide a resting place for the bugs. If desired, bugs could be reared from egg to adult without removing the snap cap.

Cultures were maintained at 80 F and 65% R.H. in an insectory, and 78 F and 50-60% R.H. in a Jamesway incubator. Bugs confined in vials were maintained under the same conditions as described, while others were kept in a room where temperature and humidity fluctuated between 65-82 F and 15-65% R.H. Temperature and humidity in the room was recorded with a Weksler hygrothermograph.

Feeding.--Colonies were fed once each week on laboratory white mice and half grown or adult white rats. The mice and rats were confined in wire cages and placed in a feeding jar. The animals were not anesthetized, but were so confined in the cage that excessive movement was difficult. They were left in the jars several hours or until exsanguinated. Bugs were fed on infected animals in the same manner (Figure 2).

Bugs kept in vials were generally fed once each week on specimens of the southern plains wood rat, Neotoma micropus micropus Baird. The rat was removed from its cage, placed in an 8 inch by 8 inch bell jar and a lid quickly placed on top. A wad of absorbent cotton, with approximately four ml of ether

added, was then placed in the jar and the lid tightly closed. When the rat became anesthetized, it was removed from the jar and inserted into a restraining cage. Cotton was packed around the rat on one end and the cage sealed with masking tape.

Restraining cages were circular, constructed of 1/4 inch mesh hardware cloth, and made in several sizes to accommodate different sized rats. A hole 1/2-inch by 1-1/2 inch was cut in the center of each restraining cage. Cages containing rats were placed in styrofoam cage holders for feeding purposes. The base of the holder was 5 inches long, 3-1/2 inches wide, and 2 inches thick. The ends were 6 inches high, 3-1/2 inches wide and 2 inches thick. A slot 2 inches deep, 2-3/4 inches wide and 1-3/4 inches deep was cut in both end pieces. A wedge was cut in one end piece for the rat's tail. A hole 1-1/4 inch in diameter and 1/2 inch deep was cut in the center of the base. Rats were positioned, in the holder, so the square opening in the restraining cage was directly over the hole in the base. A vial, containing bugs, was placed in the hole in the base; the screened top resting directly against the rat's body (Figure 3). As many restraining cages and holders as desired could be utilized.

Maintenance and Handling of T. cruzi Cultures.--Two strains of T. cruzi were maintained in the laboratory. One a Brazilian strain of unknown origin, designated Strain B, was obtained from the 4th U.S. Army Laboratory, Fort Sam Houston, Texas. The other, designated Strain L, was isolated from a wood rat, N. micropus micropus captured at Lackland Air Force Base, San Antonio, Texas in 1964.

Both strains have been grown successfully, in artificial media, for three

years. The following procedures were used: 37 g of brain heart infusion and 20 g of special Nobel agar were weighed out and added to 500 ml of distilled water in a 2,000 ml Erlenmeyer flask. The mixture was then heated slowly and stirred until dissolved. An additional 500 ml of distilled water was added and the stirring process continued until the solution was clear. Aliquots of 250 ml each were placed in 500 ml Erlenmeyer flasks. The flasks were sealed with aluminum foil and autoclaved for 15 minutes at 17 lb pressure. The media were allowed to solidify. It was then stored at a temperature of 38-42 F until used. When required, the media were placed in a water bath at 168 F for 15-20 minutes to melt. After the media had cooled, but while still warm to the touch, 50 ml of defibrinated, whole sheep blood and 10 ml of 50% dextrose solution was added to each 250 ml aliquot. The solution was thoroughly mixed and while still warm, was poured into sterile incubation flasks and vials as follows: (1) 100 ml to each 500 ml screw cap flask, (2) 25 ml to each 125 ml flask, (3) 10 ml to each large screw cap vial and (4) 6 ml to each small vial. The media were allowed to solidify without agitation.

To inhibit the growth of mold, a streptomycin-penicillin solution was added to each culture in the following amounts: (1) 15 ml to each 500 ml flask, (2) 5 ml to each 125 ml flask, (3) 2 ml to each large vial and (4) 1 ml to each small vial. The stock solution was prepared in quantity by reconstituting 10 vials of penicillin G (1 million units each) and 10 vials of streptomycin sulphate U.S.P. (1 gr each) in 1,000 ml of NaCl injection solution.

Stock cultures of the trypanosomes were subcultured at four to six week intervals. New tubes or flasks of media to be inoculated were removed from the

refrigerator and kept at room temperature, for at least one hour. The cloudy, liquid material on the surface of the old media were pipetted to the new media. The new culture media were then placed in a Jamesway incubator held at 78 F. The old media were kept for approximately 30 days in case the new cultures were contaminated. All procedures were carried out using sterile techniques.

Infection and Infectivity of Experimental Animals.--The infectivity of both strains of T. cruzi to laboratory white mice, white rats, and wood rats was investigated. Trypanosomes grown in artificial media and those passed in bug feces were used for transmission studies.

The age of experimental animals varied. However, 5-7 day old laboratory white mice and 11-28 day old white rats were normally used. Field captured wood rats of undetermined age were also utilized. Occasionally wild caught female rats delivered their young in the laboratory and these were used.

Before the wood rats were used in transmission experiments or for feeding bugs, they were checked thoroughly for evidence of natural infection with trypanosomes. The following procedures were used: rats were brought into the laboratory and anesthetized as described. A drop of tail blood was examined by phase microscopy (objective 43, ocular 10X) in cover glass preparations. One hundred fields were examined and the results recorded. If trypanosomes were seen, the rat was discarded for transmission studies. If the results were negative, 2-1/2 ml of cardiac blood was taken from the rat. One-half ml was added to culture media and incubated at 78 F for 30 days. The media was then examined for trypanosomes. The remaining 2 ml of blood was placed in a 13 x 100 mm vial and allowed to set at room temperature for two

hours. The resulting clot was broken from the side of the tube and stored in the refrigerator overnight. The next morning the blood was centrifuged at 1500 rpm for 15 minutes. The sera were removed and recentrifuged for 10 minutes. The remaining sera were placed in a 5 ml vial and delivered to the Serology Branch for complement-fixation and hemagglutination-inhibition studies using T. cruzi antigen. Laboratory reared T. gerstaeckeri and R. prolixus were allowed to feed on the rat five to 10 days after bleeding for culture and serology tests. Each lot of insects included 10 first instar nymphs or adult bugs. Two weeks after feeding the bugs were examined for trypanosomes. If any one of the above tests for trypanosomes were positive, the rat was discarded. With one exception, the above procedures were used throughout the study to determine the infection of experimental animals with T. cruzi. To further identify T. cruzi infections, animals were sacrificed and the heart fixed in formalin, dehydrated, and embedded in paraffin. Sections were cut, stained with Giemsa, and examined microscopically for evidence of Leishmania forms of T. cruzi in the muscle fibers.

Attempts to infect animals were by methods that might possibly occur in nature: (1) by bite of infected bugs, (2) contamination of skin and/or mucous membranes with infective bug feces, (3) feeding infected bugs to animals, (4) contamination of animals by direct contact, i.e., urine, sexual intercourse, and (5) congenital. Other methods, not likely to occur in nature, such as intramuscular and intraperitoneal injections were used for comparison. Infected bug feces from feeding vials were aspirated into a 0.1 ml pipette for inoculation. A 0.03 ml drop was then placed directly on the skin, eye, or mucous membrane of the

mouth. A similar amount of feces was inoculated intramuscularly and intraperitoneally using a tuberculin syringe and 0.2 ml of saline injection solution.

The number of trypanosomes, in material used for transmission studies, roughly simulated the numbers naturally passed at one defecation by a single infected adult bug. This number varied but was generally between 55 and 2850 trypanosomes per 0.01 ml of inoculum. The presence of motile metacyclic or crithidial forms of T. cruzi was evidence that the material was infective. An approximation of the number of organisms in a given amount of infected culture media were determined by haemocytometer. The level of parasitemia in animal blood and bug feces was determined by phase microscopy. A small drop of blood or feces was placed on a glass slide, diluted with saline and covered with a 12 mm cover slip. An examination was made of 100 fields and the parasitemia recorded as a given number of trypanosomes per high dry field.

An attempt was made to determine if a single trypanosome could cause infection in an experimental animal. The procedure was as follows: A small drop of naturally passed, infected bug feces was placed on a glass slide. The feces was diluted with two or three drops of normal saline injection solution and thoroughly mixed. A hollow ground slide was positioned under the low power objective of a phase contrast microscope. A drop of the mixture, small enough to be covered by the low power microscope field, was placed on the center of a cover slip. The cover slip was quickly inverted and placed over the concave part of the ground slide. The hanging drop was then examined for trypanosomes. If more than one motile form was observed the process was repeated until only one trypanosome was observed in the drop. The hanging drop was then aspirated with

a tuberculin syringe containing 0.2 ml of saline injection solution. The material was inoculated intramuscularly into 5-7 day old swiss albino mice.

Infection and Infectivity of Triatoma.--The comparative susceptibility of the three species of bugs to infection with both strains of T. cruzi was studied. In addition, the comparative effectiveness of the bugs in transmitting the infection was investigated.

Bugs were infected by allowing them to feed on animals with a predetermined circulating parasitemia. Animals previously infected, but with no visible blood parasitemia at the time of feeding were also used for comparison. Methods of restraining animals, feeding, and parasitemia evaluation have been described. All bugs were kept in contact with infected animals for 24 hours. Only those bugs that appeared to have fed to completion were kept. The bugs were examined for trypanosomes at 7, 14, and 21 days.

The determination of infection was by phase microscopy. Feces passed naturally was examined when possible. In addition, fecal samples were obtained from bugs by rectal pressure. This consists of grasping the bug between thumb and forefinger and applying pressure. A drop of feces can generally be obtained without harm to the bug. The handling of first and second instar nymphs in this manner is not feasible. It was generally necessary to puncture the rectum with a fine dissecting needle to obtain feces from these stages.

A method was devised for determining the comparative number of trypanosomes passed in adult bug feces in a given period of time. A glass rod 1/8 inch thick and seven inches long was heated two inches from one end

and bent at an angle of 20 degrees. The angled tip of the rod was repeatedly dipped in melted paraffin wax until a globular ball was formed. A bug was then suspended from the rod by pressing its thorax into the melted wax. The head of the bug was forward and the abdomen at a slight downward angle (Figure 4). A base for the rod and suspended bug was formed by cutting a 1/8 inch hole through the center of a No. 6-1/2 rubber cork. The glass rod was then inserted into the hole in the cork. The bugs were fed on a rat with a known parasitemia (Figure 5). The suspended bugs were then positioned over defecation containers. These containers were a series of five 1 ml crucibles set flush in a 1 inch x 1 inch x 8 inch styrofoam holder (Figure 6). A solid wire or nail three inches long was placed through each end of the holder. The protruding wire or nail was then imbedded in a 2 inch x 2-1/2 inch x 10 inch styrofoam base. The holder could be adjusted to the height of the bugs on the rods. One-half ml of media was added to each container. The media was the same as that used for growing trypanosomes without agar added. The bugs were maintained at 80 F and 70% R.H. and fed every two weeks on non-infected rats.

Every 24 hours the media, in the defecation chambers, was examined for trypanosomes that might have been passed in the bug feces. The media and feces, if any, were mixed thoroughly and three samples of 0.01 ml each aspirated to a glass slide. Each of the three drops were covered with a 12 mm cover slip and examined for trypanosomes. The trypanosomes were recorded as the number of trypanosomes per 100 fields as previously described. It was determined, prior to the experiment, that multiplication of trypanosomes in the media during a 24 hour period was negligible.

Membrane Feeding.--Studies on feeding behaviour and vector potential often require standard techniques in the laboratory. There are many advantages of feeding Triatoma through a membrane. The requirements for experimental animals would be reduced; infection rates in bugs could be determined more accurately, and more specific data acquired concerning the infectivity of various stages of T. cruzi grown in culture. A preliminary investigation was made on the ability of the three species of bugs to feed and ingest T. cruzi through natural and artificial membranes.

The feeder used was similar to that described by Rutledge, Ward, and Gould (1964). The bottom of the feeder had a lip over which the membrane was secured by a strong rubber band. The membranes were not stretched, but left slightly slack. A clamp and ringstand supported the feeder. The feeder was then attached, by rubber tubing, to a thermostatic controlled, circulating water bath (Figure 7). Water in the bath was maintained at 96.8 F. After the food in the feeder had warmed to the desired temperature, confined bugs were positioned against the surface of the membrane (Figure 8).

Several membranes were tested. These were wood rat skin (fresh or frozen), natural lambskin, Baudruche membrane (Bovine intestine) and parafilm "M".

Artificial membranes were washed in distilled water and dried with filter paper before use.

The food material was outdated, citrated whole human blood used as received.

Field Studies

Outdoor Cage.--Life cycle studies in the laboratory can only provide ideas on what may occur under natural conditions. To investigate the life history of T. sanguisuga texana and T. gerstaeckeri under simulated field conditions, a large outdoor cage was constructed. This cage was located on Lackland Air Force Base, Texas, in an area where wood rats and Triatoma were common and was actually built over a cactus patch where two infected wood rats had been captured.

The cage was of wood frame construction, 25 feet long, 15 feet wide and 8 feet high. The frame was set on a concrete foundation 15 inches deep and 5-1/2 inches thick. A double door entrance was provided, and the cage was covered with 18-mesh aluminum screen. The lower three feet of the cage were also covered with 1/4 inch mesh hardware cloth (Figure 9).

After construction, the cage was covered with plastic sheeting and fumigated with methyl bromide for 24 hours at the rate of 1 lb/1000 ft^3. The effectiveness of the fumigant was tested by placing confined Triatoma throughout the cage. All stages were killed at this concentration. A small mesquite tree in the cage was severely damaged, but the cacti were not harmed.

Simulated wood rat nests were built in the cage. These were fiber glass boxes 8 inches wide, 11 inches long and 6 inches deep; set flush with the surface of the ground. Small holes were drilled in the boxes for drainage. Box covers were 1/2 inch plywood, 13 inches wide and 17 inches long. A two-inch wood frame was attached to the top surface of each box cover. The area inside the frame was then filled with dirt. This arrangement held

the box covers in place and helped maintain a more even temperature inside the box (Figure 10).

Bugs, confined in plastic vials, were kept in the simulated nests. Small cages made of 1/2-inch plexiglass were also used as inserts in the nest boxes. The lid of each cage had a 2-1/2 inch x 7 inch opening covered with 32-mesh nylon screen. A strip of foam rubber 1/2-inch wide and 3/8-inch thick was glued to the inside edges of the lid to prevent the bugs from escaping. The bugs were fed once each week on wood rats.

A male and female wood rat, known to be infected with T. cruzi, were introduced into the cage at the same time the bugs were placed in the boxes. The rats fed on the cacti supplemented by lettuce and Purina laboratory chow.

Temperature and humidity, in the boxes and wood rat burrows, was recorded five days a week with a Bendix psychron fitted with a four foot extension. Temperature and humidity near ground level was recorded with a Weksler hygro-thermograph.

Field Collection of Bugs and Wood Rats.--Specimens of bugs and wood rats, for experimental purposes, were collected periodically, from wood rat dwellings.

In the San Antonio area, the southern plains wood rat, N. micropus micropus commonly constructs dwellings in clumps of prickly pear, Opuntia sp. (Figure 11).

The rat dwelling generally appears as a tangled mass of twigs, cactus stems, and debris. Entrance and exit tunnels lead to and from the nest which is generally three or four inches below the surface of the ground (Figure 12).

Debris covering the nest in the area is seldom more than 18 inches high. In south Texas the same species of rat builds dwellings up to four feet in height.

Collecting bugs from the habitat described is difficult. Equipment consists of shovel, machete, 36-inch collecting scoop, trowel, forceps, pint ice cream containers, heavy gloves, and boots. The cactus was first cleared away to facilitate examination of the nest area, then debris surrounding the nest was examined carefully for bugs. The dirt and material from any tunnel were removed with the collecting scoop. This material was spread out thinly and examined carefully. The main nest, consisting of dried grass, was then dug out and examined.

The bugs collected, were placed in ice cream cartons and returned to the laboratory. The collecting sites were in walking distance of the laboratory and no difficulty was encountered in returning the bugs alive.

Rats were collected alive by placing traps near active rat dwellings. Their activity was indicated by the presence of fresh fecal droppings and by recent "gnaw" marks on cacti. The traps used were 5 inch x 5 inch x 16 inch "Havahart" type.

Peanut butter or one-inch squares of cheese were used as bait. Both types were acceptable to the rats and no difficulty was encountered in trapping the animals where they were active.

The traps were set in the late afternoon and checked early the following morning. Two traps were generally set at each dwelling. Captured rodents were placed in small burlap sacks and returned to the laboratory for examination.

Nocturnal Flight Habits.--The flight habits of T. sanguisuga texana and T. gerstaeckeri were unknown. This phase of the study was designed to sample the adult flying population of these two species. The objectives were: (1) to determine daily and seasonal variations in flight activity, (2) to determine the sex ratio of the flying adults, and (3) to determine the infection of flying adults with T. cruzi.

Standard blacklight traps recommended by a committee of the Entomological Society of America (1964) were used in the study. As an attractant, these traps utilize one F 15T 8/BL lamp (15 watt blacklight) mounted vertically (Figure 14). The traps operate from a 110V source or they may be modified to operate with a 12V battery. A 24-hour clock timer was used to turn the traps on and off as desired.

During 1967 four traps were in operation. Two were located near a residence 16 miles northwest of San Antonio, Texas, at an altitude of approximately 850 ft. Both traps were operated five nights per week, 150 ft from the house and connected to a 110V line by extension cords. The other two traps were in operation on Lackland Air Force Base, Texas during the same period. These traps were battery operated and located adjacent to the large outdoor cage at an altitude of 740 feet. The traps were operated three to four nights per week and were timed to operate at different periods to evaluate when the bugs were most active. One trap was also operated in 1966 at the residence northwest of San Antonio.

Bugs captured each night were placed in containers and returned to the laboratory for determination of sex and infection with T. cruzi. When only females were captured they were held in vials for determination of fertility and fecundity.

Twenty-four hour temperature and humidity recordings were made at the Lackland site with a Weksler hygro-thermograph.

CHAPTER IV

RESULTS

Laboratory Studies

Mating.--Copulation, in all three species, took place with the male grasping the female and assuming a right or left dorso-lateral position. After the female became quiet, the male slowly moved caudad, rotated the genital capsule and inserted the aedeagus. The pair maintained this position throughout the copulatory period. Male bugs, when initially placed with the female, generally attempted copulation within 2-15 minutes. Females were more receptive when a blood meal was anticipated and copulation frequently took place while the female was feeding. The female of R. prolixus frequently mated again with a new male within 10 minutes after copulation. One female of this species mated with eight different males in a six-hour period. The females of T. s. texana and T. gerstaeckeri were more selective. Within a six-hour period a recently fertilized female of T. s. texana mated twice and T. gerstaeckeri three times, with different male bugs. Over a period of two years, a single pair of T. s. texana was observed in copulation 19 times. In one year, a pair of T. gerstaeckeri was observed mating 16 times. Copulation may take place within 24 hours of ecdysis. The mean and range of copulation time in minutes, for 10 pair of bugs, were R. prolixus, 39 and 15-72; T. s. texana, 19 and 9-37; and T. gerstaeckeri, 13 and 9-22.

Eggs.--The eggs of R. prolixus were a light pink when laid. After several hours they turn a lobster red. If the egg is fertile, two eye spots appear by the eighth day at 80 F and 65 percent relative humidity (R.H.). The mean size of 50 eggs was 1.8 mm x 0.9 mm. The weight ranged from 0.785 - 0.878 mg. The eggs of T. s. texana and T. gerstaeckeri were glistening white when laid. As the embryos developed they turned a light pink and the eye spots appeared by the 12th day. The mean size of 50 eggs was 1.3 mm x 0.7 mm for T. s. texana and 1.8 mm x 1.1 mm for T. gerstaeckeri. The weight ranges were 0.817 - 0.896 mg for T. s. texana and 0.832 - 1.125 mg for T. gerstaeckeri.

Oviposition.--Usually the eggs of R. prolixus were laid in batches and were fastened together and attached, by a secretion, to the surface of the styrofoam in the rearing chamber. For some unknown reason, eggs were occasionally deposited singly and without adhesive material attached. The eggs of T. s. texana and T. gerstaeckeri were deposited singly and fell to the bottom of the rearing chamber. There was no adhesive material on the eggs.

The pre-oviposition period of 10 newly emerged females of each species which had fed, mated once, and been held at 80 F and 65 percent relative humidity, was 8 - 14 days for R. prolixus, 12 - 23 days for T. s. texana, and 11 - 19 days for T. gerstaeckeri.

Fecundity and Longevity of Adults.--The fecundity and longevity of adult bugs are important factors in the species reproductive potential. Egg production and longevity were determined at both fluctuating and constant levels of temperature and humidity.

Ten pairs of each species were placed in individual vials. Five pairs of each species were held at 65 - 82 F and 15 - 65 percent relative humidity and the other pairs were held at 80 F and 65 percent relative humidity. Only eggs from which living nymphs emerged were considered as hatching. The data are presented in Table I - II.

There was considerable variation in the number of eggs laid by females under any condition. Female T. s. texana and T. gerstaeckeri laid a higher mean number of eggs at fluctuating than at constant temperatures while R. prolixus was the opposite. The percent egg hatch, of all three species, was higher at the constant temperature. In all three species, the percent egg hatch was generally declined if the male bug died before the female. For example, T. s. texana female A (Table I) lived 813 days and laid 1066 eggs with a hatch of 521 (49%). However, from ecdysis on 1 June, 1965 until 8 September, 1966, 505 eggs were laid and 356 (70%) hatched. On 28 September, 1966, the male died. From 8 September, 1966 to 7 November, 1966, 161 eggs were laid and 102 (63%) hatched. From 7 November, 1966 to 12 March, 1967, no eggs were laid. From 12 March, 1967 to 3 July, 1967, 282 eggs were laid and none hatched. On 10 July, 1967, a new male was placed with the female. From 16 July to 20 August, 1967, 118 eggs were laid and 63 (53%) hatched. The female died 21 August, 1967. The same cycle occurred in individuals of T. gerstaeckeri and R. prolixus. This would indicate that one fertilization is not sufficient for the life of the female, especially if she lives for at least a year. Most females laid eggs throughout their lives. Egg production in T. s. texana and T. gerstaeckeri decreased in October and November and few eggs were laid until the

following March. This correlated remarkably well with egg production under natural conditions. In the laboratory, R. prolixus produced eggs throughout the winter months.

The mean life of R. prolixus and T. s. texana females was greater at the fluctuating temperature (Table I). Triatoma gerstaeckeri male and female longevity was virtually the same. At a constant temperature (Table II), the male of all three species lived longer than the female. This may indicate that males are less hardy than females under variable conditions. The male and female of T. s. texana lived almost twice as long as the other two species.

Egg Production of Virgin Females.--Five virgin females of each species were placed in individual vials and held at 80 F - 65 percent relative humidity. All three species laid eggs that were normal in number and appearance. However, after a few days the eggs collapsed and none ever hatched. One female R. prolixus in this group lived 882 days. This was the longest that any bug survived.

Number of Eggs Laid After One Copulation.--The ability of a female bug to mate once and lay viable eggs for several months would be of great value in the survival of the species. To test this, five virgin females of each species were isolated in individual vials, mated once to virgin males and held at 80 F - 65 percent relative humidity.

There was considerable variation in the number of eggs laid by individual females (Table III). The mean percent egg hatch and total number laid were less than for male and female bugs kept together (Table I - II). As the female aged there was a decrease in the percent egg hatch. For example,

T. s. texana females A-D (Table III) lived longer, but had a lower percent egg hatch than the females who died much earlier. This was also true in T. gerstaeckeri G-J, and R. prolixus N-O. The percent egg hatch in the first 90 days was invariably higher than any thereafter. If the female survived for more than a year, fertile eggs were not laid. This was comparable to the condition found in females when the male died first.

Fecundity and Longevity of Unfed Adults.--The ability of bugs to lay viable eggs without a blood meal would be a significant survival factor in nature. Five pair of newly emerged, virgin male and female bugs of each species, were placed together in individual vials and held at 80 F - 65 percent relative humidity. The proboscis of each pair was clipped to prevent the possibility of feeding on each other.

It is evident (Table IV) that a few viable eggs were laid by females, of each species, without a blood meal. This may be due to nutritional elements transferred from the fifth instar nymph to the adult at ecdysis. The viable eggs were generally among the first laid, Unfed males survived longer than unfed females.

Development Period from Egg to Adult.--Twenty one day old eggs, of each species, were isolated in individual vials and the emerging nymphs reared to the adult stage. The eggs were held at 65 - 86 F and 18 - 68 percent relative humidity, and at 80 F - 65 percent relative humidity. Eggs and nymphs were examined daily except weekends. Table V indicates the minimum, maximum, and mean time for incubation of eggs and development of each instar.

The developmental period varied greatly among individuals of T. s.

texana and T. gerstaeckeri at both temperatures. Rhodnius prolixus showed much less variation. The mean developmental period for T. s. texana was 387.8 days at the fluctuating temperature and 322.1 days at the constant temperature. The mean developmental period for T. gerstaeckeri was 361.9 and 213.9 days, respectively, and that for R. prolixus was 126.1 and 112.8 days. The first two adult T. s. texana to emerge were males. The first four T. gerstaeckeri were males and the first two R. prolixus were females.

Feeding Habits.--Observations were made on various feeding habits that might contribute to the survival and vector effectiveness of the bugs.

When approaching the host, T. s. texana and T. gerstaeckeri were more cautious than R. prolixus. Triatoma sanguisuga texana was especially shy in the nymphal stages. Triatoma sanguisuga texana and T. gerstaeckeri preferred to feed with at least the rear legs in contact with a solid surface. They rarely crawled on the host animal except under crowded conditions. Rhodnius prolixus, on the other hand, approached the host boldly and did not hesitate to crawl over the host's body while feeding. This habit is undoubtedly important in the transfer of T. cruzi by fecal contamination. Bugs, of all stages, preferred to feed in subdued light or darkness. However, when hungry they fed in bright light.

It was observed, in other experiments, that T. s. texana was difficult to rear in individual vials. Heavy mortality generally occurred among first instar nymphs. A series of tests were conducted to compare the feeding of first instar nymphs on various host animals, and to determine if one feeding was sufficient for molting.

Twenty, unfed nymphs, one to two weeks old, were placed in a feeding jar and held for 24 hours at 80 F and 65 percent relative humidity. A host animal was placed in the jar and left for 24 hours or the host was confined and the bugs placed in direct contact with the animal. Bugs that fed were held until they molted or died. This procedure was repeated with each species of bug, each host animal, and at 65 - 86 F and 25 - 60 percent relative humidity.

Rats, adult mice, and the squirrel were confined in restraining cages in the jars. The armadillo was restrained and the bugs fed directly. The remaining host animals were placed free in the jar. Nymphs offered human blood (my own) were confined in small ointment tins covered with nylon stocking and secured to the underside of the forearm by adhesive tape. Since the test results were similar at both conditions, the data for the 40 nymphs are presented together in Table VI.

The bugs preferred mammalian blood but did feed on all the host animals tested. Triatoma gerstaeckeri and R. prolixus fed better than T. s. texana on all host animals except engorged bugs. The largest number of T. s. texana nymphs fed on the adult white mouse. Triatoma gerstaeckeri nymphs fed best on the armadillo and those of R. prolixus on human blood.

There was considerable variation in the number of nymphs that molted (Table VI). Only two of the T. s. texana nymphs molted following one feeding. In T. gerstaeckeri and R. prolixus, fed on various mammals, the percent molting was 62.5 - 87.5 and 84.3 - 95 respectively. For the same two species fed on hosts other than mammals, the percent molt was 0 - 54 and 0 - 87. The highest percent (83.8) of molting of T. gerstaeckeri nymphs occurred in those

fed on the ground squirrels and the highest percent (95) of R. prolixus in those fed on human blood. The only apparent refractory blood was that of the snake, Thamnophis sp., to R. prolixus. Sixteen of 18 nymphs that fed, died within 48 hours and the two survivors molted to the second instar.

Feeding and Defecation Times.--The period of time a bug is in contact with its host may be important in the chance contaminative transmission of T. cruzi. This is particularly true if the bug, while feeding, defecates on or near the host. An experiment was conducted to gather data regarding feeding and defecation times. The results are presented in Table VII.

It was observed many times that individuals of the three species, quickly moved away from the host after feeding. It was arbitrarily decided to record only defecations that occurred within a period of two minutes. Bugs that defecated after that time would be less likely to contaminate their host.

The mean feeding times for T. s. texana and T. gerstaeckeri were about the same in all stages. In general, these two species fed more than twice as long as R. prolixus. The mean feeding time for adult females was longer than that of males in all three species. The percent defecations (56 - 88.8) in all stages of R. prolixus were considerably higher than in T. s. texana (0 - 26.6) or T. gerstaeckeri (0 - 30). The adult males of T. s. texana and T. gerstaeckeri did not defecate within two minutes of feeding. In R. prolixus males, the percent defecation (56.6) was less than for females (77.5).

It is concluded that R. prolixus, in all stages, may contaminate its host with feces to a much greater extent than T. s. texana or T. gerstaeckeri.

The fourth and fifth nymphal instars and adult females of T. s. texana and T. gerstaeckeri are potentially better fecal contaminators than the other stages (Table VII).

Since first instar nymphs of T. s. texana did not molt after one feeding an experiment was conducted to determine how many times they fed before molting. Six unfed, first instar nymphs, were placed in individual vials and offered food daily until they molted or died. Five molted to the second instar. Of these, two fed four times, one fed five times, and two fed seven times. This was not repeated for every instar, but many observations indicate this species feeds several times in each instar. This would be an important factor in the opportunity to transmit T. cruzi to susceptible hosts over a period of time.

It has been shown (Table VI) that first instar nymphs of all three species will feed on cold-blooded vertebrates. It was determined that bugs of all stages will take at least one blood meal from a toad Bufo sp. Toads were selected because they frequently live in the same general habitat as the bugs and were occasionally found hibernating near wood rat dwellings. The question was, would the bugs complete their life cycle feeding on the blood of a toad? An attempt was made to answer that question.

Five, unfed, first instar nymphs of each species were placed in three separate vials and fed weekly. Unfortunately, the toad died after 167 days and since it was the beginning of the winter season an additional specimen could not be located. When the toad died, one second and one third instar T. s. texana and one fourth instar T. gerstaeckeri were alive. The last nymph of R. prolixus

died in the fourth instar.

Coprophagy.--A series of experiments were conducted to determine if bugs would be attracted to and feed on freshly voided bug feces. Twenty, unfed, first instar nymphs of each species were placed in feeding jars and held at room temperature for 24 hours. A concave cell slide was placed in the jar and filled with bug feces. As the starved bugs moved about they would occasionally pass the feces, extend their proboscis and probe. When feces, warmed to 90 F was added, the bugs were more direct in their approach to the possible food source. The slide and feces was left in the jar for 24 hours. At the end of that time the bugs were examined for any visible evidence of feeding. The above procedure was repeated four times within a period of four months. There was never indication that bugs of any stage fed on feces.

Amount of Blood Ingested at One Feeding.--The amount of blood taken at a single feeding was determined for bugs of all stages. Ten individuals of each stage were weighed with a Model G Cahn Gram Electrobalance before and after feeding. The mean weight of each stage was recorded to 0.01 mg (Table VIII). The percent gain was obtained by dividing the amount of blood ingested, by the weight of the unfed bug. First instar nymphs were fed and weighed at one week of age and the other stages from one to two weeks after ecdysis. It was difficult to obtain exact blood meal weights for R. prolixus, as they defecate while feeding or shortly after.

Rhodnius prolixus, in all stages, ingested more blood in relation to their size, than the other two species. The adult females ingested more blood than the adult males. One reason for this is that the female is generally

larger than the male (Figure 4). The highest percent gain was found in first instar nymphs of T. s. texana and T. gerstaeckeri and the third instar of R. prolixus. The greatest volume of blood ingested was in fifth instar nymphs of T. s. texana and R. prolixus and in the adult female of T. gerstaeckeri.

Infection of Experimental Animals.--The purpose of this phase of the study was to: (1) find out if there was a difference in the virulence of our two strains of T. cruzi to laboratory animals; (2) determine the parasitemia, if any, produced by various routes of inoculation and, (3) determine the appearance and persistence of the parasitemia in the circulating peripheral blood.

There was very little difference in the virulence of the two strains to laboratory animals (Table IX). This was true of organisms grown in culture or passed in bug feces. Animals would occasionally show symptoms of infection, such as roughening of the fur, lethargy, and retarded growth, but death in infected animals was not common. Not all animals became infected though inoculation procedures were the same. Intramuscular inoculation produced a greater number of infections than the oral route.

Table X indicates the results of infecting experimental animals by various routes of inoculation. The inoculum was the Lackland strain in bug feces. It was evident that the bite of infected bugs did not produce infection. Oral contamination and ingestion of bugs caused the largest number of infections among those routes of inoculation that might occur in nature. Rats could become infected by eating one infected bug. Only one rat became infected by placing the infected feces directly on the unbroken skin and a low parasitemia developed. Application of feces directly into the eye also gave a low infection

rate and parasitemia. Intramuscular and intraperitoneal inoculation produced high infection rates and relatively higher parasitemias. Inoculation of feces directly into the stomach, by tube, produced infection in two wood rats that were 41 days old. No trypanosomes were observed in direct blood smears; however, blood cultures were positive 122 days post inoculation. There was no observable difference in the infectivity of feces from the three species of bugs.

Uninfected laboratory animals kept in direct contact with infected litter mates never became infected. This was also observed in wood rats reared in the outdoor cage. In July 1965, an infected pair of rats was placed in the outdoor cage; at the end of nine months, 17 rats were recovered. None of the 15 offspring of the original pair was infected. This would indicate that infection by direct contact, i.e., urine, feces, and sexual intercourse is rare in nature.

Eight pregnant white mice were inoculated intramuscularly with culture forms of T. cruzi. Two of the females became infected. None of their offspring was infected. Two infected female wood rats gave birth to young in the laboratory and these were not infected.

In animals less than 30 days old, the parasites were observed in the blood 5 to 14 days following inoculation. The parasitemia developed more rapidly in animals inoculated intramuscularly. The course of the infection was characterized by a rise in parasitemia that reached a peak at an average of 21.8 days in laboratory animals and 36 days in wood rats. The parasites rapidly disappear from the blood within 10 days after reaching a peak. In a wood rat the maximum time that trypanosomes were detectable in the peripheral blood was 47 days after they were first observed. The maximum number of

circulating trypanosomes observed in any animal was 700/100 high dry fields. It was extremely difficult to produce an observable parasitemia in animals more than 40 days old. In fact, it was difficult to infect older animals at all.

Ten, five-day old white mice were inoculated intramuscularly with 0.2 ml of saline that originally contained one motile trypanosome. Thirteen days later one mouse had a parasitemia of one trypanosome per 100 high dry fields. The parasitemia never increased and disappeared after 21 days. This was the only mouse that became infected.

Susceptibility of Bugs to Infection.--A comparison was made of bug susceptibility to the two strains of T. cruzi. The results are indicated in Table XI.

The bugs were fed on wood rats with a circulating parasitemia of one to five trypanosomes per high dry field. First and second instar nymphs were examined for evidence of infection at 21 days and the other stages at 7, 14, and 21 days. Bugs found uninfected at 21 days were held for further examination.

There was little difference in the susceptibility of the three species to the Brazilian strain of T. cruzi. Triatoma sanguisuga texana and T. gerstaeckeri were more susceptible to the Lackland strain than R. prolixus. The adult females were more susceptible to both strains than the adult males. There was no definite pattern to nymphal susceptibility. It was apparent that not all bugs became infected after feeding on an infected host. Five adult females, of each species, originally infected with the Brazilian strain, were examined 90 days later for evidence of infection. All, except one R. prolixus, remained infected. The same result was obtained with the Lackland strain,

except two R. prolixus were negative. Ten, first instar nymphs of R. prolixus were infected with the Brazilian strain and reared to adults. Of eight, that reached the adult stage, five were still infected. With the Lackland strain, five of nine were infected at the adult stage.

Table XII indicates the results of feeding bugs on wood rats with no observable circulating parasitemia in the peripheral blood. The rats had been infected with the Lackland strain, 180 days prior to the experiment. When compared with Table XI, it is clear there was a decrease in the number of infections in bugs fed on rats with no observable parasitemia. Again adult females were more susceptible to infection than adult males.

The relative number of trypanosomes passed daily in adult bug feces was determined. The bugs were suspended on glass rods, fed on a white rat infected with the Lackland strain and held at 80 F and 65 percent relative humidity. The rat had a parasitemia of five trypanosomes per high dry field. Results are given in Table XIII.

It was again apparent that R. prolixus defecated more often than T. s. texana or T. gerstaeckeri. Trypanosomes appeared in the feces of R. prolixus on the fifth day and in T. s. texana and T. gerstaeckeri on the eighth day. All three species passed the largest number of trypanosomes and volume of feces during the first 24 hours after feeding. There was considerable variation in the number of trypanosomes passed in individual bug feces.

Membrane Feeding.--The comparative ability of the bugs to feed through various membranes and to become infected with T. cruzi was investigated. The bugs were placed in plastic vials under the feeder and the feeder

and bugs covered with a black cloth to reduce light. The bugs were in contact with the membrane from one to two hours. Two ml of culture medium, containing approximately 150 thousand trypanosomes per ml were mixed with the food before placing it in the feeder. The food material was maintained at 97 F and the ambient temperature was 70 - 75 F. Trypanosomes were still swarming in the food at the end of each feeding period.

The wood rat skin gave the best feeding results followed by the Baudruche membrane (Table XIV). Rhodnius prolixus fed better than T. s. texana or T. gerstaeckeri.

Adult bugs appeared to feed better than nymphs. The low percent feeding may have been caused by packing of blood cells against the inner surface of the membrane. For some unknown reason, the infection rate was very low in bugs that fed. For example only one nymph and one adult of T. s. texana became infected. The greatest number of infections were in R. prolixus. This was just the opposite from what occurred when the bugs fed on natural hosts (Table XI). Of the total infections obtained, 15 (78.8%) were in bugs that fed through the wood rat skin. The remaining 21.2 percent fed through the Baudruche membrane.

Field Studies

Developmental Period Egg to Adult.--Ten, newly emerged, first instar nymphs of T. s. texana and T. gerstaeckeri were placed in individual vials in the outdoor cage in October, 1965 and March, 1966. Observations were made on their development to the adult stage. The developmental period of the various

instars varied considerably depending on the time of year they emerged (Table XV).

First instar nymphs, that emerged in October, remained in that stage until the following March or April. Triatoma sanguisuga texana reached the fourth instar and T. gerstaeckeri the fifth in July or August. Both species remained in this instar until the following spring. Triatoma gerstaeckeri then emerged as adults in April and May and T. s. texana molted to the fifth instar. One fifth instar T. s. texana emerged as an adult in September, approximately 715 days after hatching. Two other fifth instars are still in that stage after 289 days. They will probably overwinter and emerge in the spring after approximately 2-1/2 years. First instar nymphs of both species that emerged in the spring reached the fourth and fifth instars at virtually the same time as the nymphs that emerged in the fall. One of 10 nymphs of T. gerstaeckeri that emerged in July reached the fifth instar in November and emerged the following March. This was the shortest time (280 days) that any individual took to complete its life cycle under field conditions. Numerous variations, depending on temperature and availability of food, undoubtedly occur in the life cycle of the bugs. It is suggested that, with readily available food, the life cycle of T. gerstaeckeri in southwest Texas is 9 to 14 months and that of T. s. texana 24 to 30 months.

Comparative Fecundity and Longevity of Adults.--Three pair of T. s. texana and T. gerstaeckeri were placed in vials in the outdoor cage and observations made on their fecundity and longevity.

Triatoma sanguisuga texana laid fewer eggs in the field (Table XVI)

than in the laboratory (Table I - II). The number laid by T. gerstaeckeri was about the same. The largest number of eggs laid in one season was 803 by T. gerstaeckeri and 465 by T. s. texana. The percent of eggs that hatched was less under field than laboratory conditions. A comparison of monthly egg production indicates considerable variation in both species (Table XVII). The greatest number of eggs was laid in April, May, and early June. In both species, the highest percent egg hatch was in May, when the average minimum temperature in the boxes was 70.9 F and the maximum 84.7. Egg hatching decreased in late June, July, and August when the average maximum temperature was over 90 F. During September the percent egg hatch increased when the average maximum and minimum temperatures were similar to those in May (Table XVII). In our study, humidity was not considered to be a factor in the percent egg hatch. The daily outside fluctuation in relative humidity was 20 - 60 percent in May; this was very close to the daily fluctuations in July and August. The relative humidity in the boxes ranged from 30 - 90 percent with reading rarely below 40 percent.

Triatoma sanguisuga texana did not survive as long in the field as in the laboratory. Conversely, T. gerstaeckeri lived slightly longer in the field. Rhodnius prolixus were not used in field experiments to any extent because of the danger of their escaping. One experiment was conducted to determine if this insect would survive the winter in this area. A male and female were placed in separate vials in the outdoor cage in November and observations made on their behavior. Both specimens survived the winter in good shape. During periods of cold weather they fell to the bottom of the vial and appeared to be dead.

However, when warm periods occurred they revived and moved about. No eggs were laid from November to 30 April. The pair were mated in May and produced viable eggs.

Survival with One Feeding.--The ability of the bugs to survive the winter on one feeding was determined. Individuals of all stages were fed once, and placed in the outdoor cage on 1 November 1966.

Most individuals survived the winter on one feeding. The greatest mortality occurred in first instar nymphs. As the temperature rose in the spring the bugs began to die. Fifth instar nymphs survived the longest and one T. s. texana lived for 385 days. The males of T. s. texana lived longer than the females but T. gerstaeckeri females lived longer than the males (Table XVIII).

Intraspecific Parasitism.--All three species of bugs were observed feeding on their own kind in laboratory colonies. This practice was more common among T. s. texana than in the other two species. First and second instar nymphs were observed to feed in this manner more readily than other stages. It was never observed among adult insects when the bugs had freedom of movement.

The relative importance of this habit in the survival of first instar nymphs in nature was determined. Fifty, first instar nymphs of T. s. texana and T. gerstaeckeri were placed in separate boxes in the field cage. Material from rat nests was placed in the boxes to provide shelter. At two to three week intervals, five engorged fourth and fifth instar nymphs, infected with T. cruzi, were placed in the boxes and left until other bugs were added. At the end of

one year the material in the boxes was examined for surviving nymphs.

Eleven (22%) of the T. s. texana nymphs were alive at the end of one year; eight were in the second instar and three in the third. Eight (16%) of the T. gerstaeckeri were alive; seven second instars and one third instar. The surviving nymphs had apparently imbibed only haemolymph and none were infected with T. cruzi.

The various stages of Triatoma are particularly vulnerable to being fed upon during the process of ecdysis. Figure 14 shows first instar nymphs of T. gerstaeckeri feeding on an emerging adult of R. prolixus. In this case the nymphs imbibed only haemolymph. However, it is possible that the mouthparts of larger nymphs or adults could penetrate the soft exoskeleton and ingest blood from the donor's gut.

Field Collections.--The primary purpose of collecting Triatoma and wood rats was to obtain specimens for experimental purposes.

From March, 1965 through October, 1967, 142 wood rat dwellings were examined for nymphs and adults of T. s. texana and T. gerstaeckeri. There was no apparent seasonal fluctuation in the nymphal population density and the summarized data and observations are presented here.

Eighty-five (68.5%) of the dwellings examined contained a total of 213 nymphs and 16 adults of the two species. One hundred twenty-three nymphs and nine adults were T. s. texana. Ninety nymphs and seven adults were T. gerstaeckeri. The mean number of bugs collected per infested dwelling was 2.5 with a range of 1 - 22. Nymphs of all instars, except the first, were collected at every season of the year. First instars were difficult to locate in the

debris and were rarely found. Over the collecting period, four adults were collected from dwellings in April, three in May, one in June, two in August, and three in September.

The bugs were rarely found in the rats nests. The preferred habitat appeared to be in cracks and crevices in tunnels and runways leading to the nest or in debris covering the nest. Nymphs were occasionally found under logs and debris several hundred feet from the nest of any known host. The condition of most of the bugs indicated they had not fed for sometime. This was true even when the bugs were within a few feet of an occupied nest.

The 229 Triatoma collected were examined for infection with T. cruzi-like organisms and 79 (30.1%) were infected; 33 T. s. texana and 46 T. gerstaeckeri. There was a marked difference in the ratio between infected nymphs and adults. Twenty-eight (22.7%) of the 123 T. s. texana nymphs collected were infected and five (56.5%) of nine adults. Forty-two (46.6%) of 90 T. gerstaeckeri nymphs collected were infected and four (57.1%) of seven adults.

From 1 March, 1965 to 29 September, 1967, 56 wood rats were trapped. Twelve (21%), five females and seven males, were infected with T. cruzi. Three (25%) of the 12 infected rats had a demonstrable circulating parasitemia by direct blood examination. The three rats with a circulating parasitemia were estimated to be between four and six weeks old. It was obvious that any survey, for T. cruzi, based on direct blood examinations alone would be highly inaccurate.

The rats were active throughout the year. Young rats were observed in February and September. From observations in the field cage, there were

probably three or four litters per year. The usual number of young is two. Several rats may occupy one large cactus patch (Figure 11).

Nocturnal Flight Habits.--During the period 1 June - 31 September, 1966, and 1 April - 31 September, 1967, 698 adult T. gerstaeckeri were collected in blacklight traps at two different sites in Bexar County, Texas. During the same period only 13 adult T. s. texana were collected. The data presented apply to T. gerstaeckeri unless otherwise noted.

The first bugs were collected at Lackland AFB, Texas; a male on 24 April and a female on 11 May. Collections from the residence in northwest Bexar County were recorded weekly, and the first bugs, all males, were collected 1-7 May. The first females were collected 25 May - 1 June. The last bugs were collected during the last week of September at both sites. During 1967, the largest flights occurred in May, June, and July (Table XIX). Traps were not available during May, 1966. However, large flights occurred in June, July, and August of that year (Table XX). Considerably more males than females were collected at the residence than at Lackland AFB (Table XX). In contrast, more females were taken at Lackland AFB (Table XIX). In the spring males appeared approximately two weeks before the females. The maximum number of bugs collected in a single trap in one night was 15 on 1 June, 1967, at Lackland AFB. The night was clear with a maximum temperature of 88 F and a minimum of 72 F during the collection period. Nine bugs were collected on 4 May when the weather was cloudy with a light drizzle and fog. The maximum flight activity occurred when the average minimum-maximum temperature was between 66.5 - 95 F (Table XXI). The maximum summer

temperatures in southwest Texas, frequently exceed 100 F. From 8 May - 5 September, 1967, a hygro thermograph at the Lackland AFB site recorded 56 days when the temperature equaled or surpassed that figure. Evening temperatures frequently remain above 90 F, two or three hours after sunset. Flight activity was determined for the periods 6:00 - 12:00 P.M. and 12:00 - 7:00 A.M. Forty-one (89.1%) of the bugs were taken after midnight and five (10.9%) before midnight. The minimum-maximum temperature during the 12:00 - 7:00 A.M. collection periods was 76.4 - 84 F (Table XXII). Unfortunately, timers were not available for the traps in April or May so data for these months are not available. However, observations of traps at 8:00 P.M. on eight occasions, in April and May indicated no bugs were captured to that time. There was no observable relation between light intensity and flight of the bugs.

Many factors other than temperature, undoubtedly stimulate flight in Triatoma. The general appearance of the captured bugs was similar to laboratory specimens that had not fed for approximately 14 - 21 days. Only two of the 698 bugs captured had recently fed. Ten unfed females were taken from traps and kept in individual vials until they died. The mean survival period was 13 days with a range of 4 - 22. During this time four of the females laid 1 - 4 egg each. Fifteen females collected without males were fed and placed in individual vials. Nine laid viable eggs indicating they had been fertilized. Two died without laying eggs, and four laid eggs that were not fertile.

Of 698 bugs examined for T. cruzi-like organisms, 544 (77.9%) were infected. There was considerable variation in the infection rate in male bugs at the Lackland AFB and residence site, while the infection rate in females

was similar (Table XIX). At the residence site, infection rates in male bugs was slightly higher in 1967 than 1966, while that in females was about the same (Table XX). The infection rate in male and female bugs was about the same at the Lackland AFB site (Table XIX).

Wood rat dwellings were abundant near the Lackland AFB site, while they were uncommon at the residence site. However, armadillos were abundant near the residence in northwest Bexar County. Five armadillos were captured and examined for trypanosomes and 2 (40%) were infected. Both infected animals had circulating parasitemias of approximately 200+ trypanosomes per high dry field. This is in contrast to an infection rate of 21 percent and maximum parasitemia of one trypanosome per high dry field in wood rats at the Lackland AFB site. No specific data were kept on the number of trypanosomes in bug feces from the two sites. However, the highest number observed in a single bug from the Lackland AFB site was 37 per high dry and 150+ from the residence site.

Samples of blood were taken from two adults, two children, two dogs, one cat, and one pony at the residence site. The humans reportedly had been bitten many times by Triatoma bugs over a six year period. None of the blood samples was positive for T. cruzi.

The small number of T. s. texana collected in the survey is surprising since this species occurs in wood rat dwellings in approximately the same proportion as T. gerstaeckeri. The stimulus that produces flight activity in T. gerstaeckeri may not affect T. s. texana or they are not readily attracted to blacklight.

CHAPTER V

SUMMARY AND CONCLUSIONS

The objective of the investigation reported here was to compare the biology and vector capability of two Texas species of Triatoma with an effective South American vector of American trypanosomiasis. From this study a clearer understanding of the ecology of the vectors and the factors that may or may not contribute to vector effectiveness was obtained.

The number of eggs laid by individual bugs varied considerably under any condition. In the laboratory, the maximum number laid was 1,066 by Triatoma sanguisuga texana, 1,069 by Triatoma gerstaeckeri, and 702 by Rhodnius prolixus. In the field the maximum was 465 by T. s. texana and 803 by T. gerstaeckeri. Rhodnius prolixus was not used in field studies to any extent. In the field, egg production tapered off and ceased during October and November and began again in March and April. The largest number of eggs were laid in April, May, and June. This same cycle occurred in the laboratory with T. s. texana and to a lesser degree with T. gerstaeckeri. Rhodnius prolixus laid eggs throughout the year in the laboratory. The percent egg hatch was less in the field than in the laboratory with T. s. texana and T. gerstaeckeri. The greatest percent egg hatch, in the field, was in May when the average minimum temperature was 70.9 F and the maximum temperature was 84.7 F. Egg hatchability decreased in late

June, July, and August when the average maximum temperature was over 90 F. In September the egg hatch increased again when the temperatures were similar to those in May.

There were fewer number and less viable eggs laid after only one copulation than when male and female bugs were together continually. One copulation was not sufficient to produce viable eggs for the life of the female. The percent egg hatch was greater in the first 90 days after mating. Viable eggs were occasionally laid without a blood meal. This may be due to nutritional elements transferred from the fifth instar nymph to the adult at ecdysis. Virgin females did not produce viable eggs.

As might be expected the developmental period from egg to adult varied with the temperature. At fluctuating temperatures, within 65 - 86 F, the average incubation period was 388 days for T. s. texana, 362 for T. gerstaeckeri, and 126 for R. prolixus. At a constant temperature of 80 F, the average periods respectively, were 322, 214, and 112 days. In the field, developmental periods were related to temperature and the time of year eggs were deposited. It is believed that the life cycle of T. s. texana in southwest Texas was 24-30 months and that of T. gerstaeckeri was 9-14 months. Each species passed through five nymphal instars both in the laboratory and in the field.

The longevity of adult bugs varied greatly under the same environmental conditions. In the laboratory the maximum longevity in days for male and female respectively, were: T. s. texana 787 and 813; T. gerstaeckeri 417 and 412; and R. prolixus 458 and 882. In the field the same

data were: T. s. texana 680 and 585; and T. gerstaeckeri 398 and 378. The mean survival time of male and female T. s. texana was almost twice as long as the other two species. Most individuals of T. s. texana and T. gerstaeckeri will survive the winter in this area on one feeding. Among nymphs the fifth instars survived the longest and one T. s. texana lived for 385 days. A male and female R. prolixus survived the winter, mated and produced viable eggs. It is concluded that if R. prolixus is introduced into this area of Texas, they will survive and reproduce.

Observations were made on feeding habits which might contribute to the survival and vector potential of the bugs. Triatoma sanguisuga texana and T. gerstaeckeri preferred to feed with at least the rear legs in contact with a surface other than the animal and rarely crawled on the host animal. Conversely, R. prolixus did not hesitate to crawl on the host's body in an effort to initiate feeding. This habit, coupled with the fact that R. prolixus defecates more frequently, and in greater quantities, makes it a more efficient contaminative transmitter of T. cruzi than the other species.

All three species preferred to feed on mammalian blood but would also feed on cold blooded vertebrates and birds. In the laboratory the preferred host of T. s. texana was adult white mice. Triatoma gerstaeckeri fed best on the armadillo and R. prolixus on human blood. All three species were fed as first instar nymphs on a toad; T. s. texana reached the third instar and T. gerstaeckeri and R. prolixus the fourth. It is concluded that in nature the bugs may survive for long periods of time by feeding on cold blooded vertebrates.

Nymphs of all three species would occasionally feed on their own kind. The practice was most common among individuals of T. s. texana. In the field, first instar nymphs of T. s. texana and T. gerstaeckeri fed, molted, and survived for one year on haemolymph from fourth and fifth instar bugs. Since only haemolymph was ingested, no transfer of T. cruzi from infected to non-infected bugs took place. The various stages of Triatoma are particularly vulnerable to being fed upon during the process of ecdysis. It is possible that the mouth parts of large nymphs may penetrate the soft exoskeleton at this time and ingest blood from the donor's gut.

The practice of coprophagy was never observed and it is highly unlikely that it is of any epidemiological significance in the maintenance of T. cruzi in nature.

A high percentage of first instar nymphs of T. gerstaeckeri and R. prolixus molted after one feeding. Triatoma sanguisuga texana nymphs generally fed four to seven times before molting. If the number of times a bug fed during its lifetime were a major criterion for T. cruzi transmission, T. s. texana would be an excellent vector.

Triatoma sanguisuga texana and T. gerstaeckeri in all stages fed almost twice as long as R. prolixus. The mean feeding time for females was longer than males in all three species. Rhodnius prolixus, in all stages, defecated on or near the host much more frequently than the other two species. The adult males of T. s. texana and T. gerstaeckeri did not defecate while feeding. The fourth and fifth nymphal instar and adult females are potentially better fecal contaminators than the other stages.

Rhodnius prolixus, in all stages, ingested more blood in relation to their size than the other two species. Adult females ingested more blood than adult males. The greatest volume of blood actually ingested was by fifth instar nymphs of T. s. texana and R. prolixus, and by adult females of T. gerstaeckeri.

The Brazilian and local strain of T. cruzi was of low virulence to experimental animals. Low parasitemias were produced regardless of the method of inoculation and deaths in experimental animals rarely occurred. Intramuscular inoculations produced the most infections and highest parasitemia in animals. Not all animals became infected by any route of inoculation, though procedures were the same. Direct oral contamination and ingestion of bugs, produced the greatest number of infections among those routes of inoculation that might occur in nature. Infection by direct contact, i.e., urine, feces, and sexual intercourse, was never demonstrated and the transmission of T. cruzi by these methods is probably rare in nature. Transmission was not accomplished by the bite of infected bugs. It was demonstrated that a rat could become infected by feeding on one infected Triatome bug. An infection was produced in a white mouse by the injection of a single trypanosome.

Parasites were observed in the peripheral blood 5 to 14 days following inoculation and peaked at an average of 21.8 days in laboratory animals, and 36 days in wood rats. Parasites rapidly disappeared from the blood within 10 days after reaching a peak. The maximum time that trypanosomes were detectable in the blood of a wood rat was 47 days. It was extremely difficult

to produce an observable parasitemia in animals more than 40 days of age. In the field, observable circulating parasites were found only in young wood rats that were estimated to be between four and six weeks of age.

It is postulated that the wood rat Neotoma micropus micropus becomes infected with T. cruzi primarily by ingesting infected bugs. The infection is most likely acquired when young, curious, newly weaned rats wander about and feed on infected bugs found near the nest. It was obvious that any surveys for T. cruzi based only on the direct observation of circulating parasites in the peripheral blood would not be accurate.

There was little difference between the three species of bugs in their susceptibility to infection with the Brazilian strain of T. cruzi. Conversely, T. s. texana and T. gerstaeckeri were more susceptible to the Lackland strain than R. prolixus. There was no discernible pattern to nymphal susceptibility. However, adult females were more susceptible to both strains than males. Not all bugs became infected when they fed on an infected host. The percent infection rate was higher in bugs which fed on rats with an observable parasitemia than in those without. The infection persisted for life in T. s. texana and T. gerstaeckeri. Over a period of time a few individuals of R. prolixus lost their intial infection. It is concluded that the effective use of non-infected bugs, as a diagnostic tool for T. cruzi infections, as described by Maekelt (1964), would be greatly influenced by the degree of parasitemia in the host at the time the bugs fed and by the sex of adult bugs used. Since first instar nymphs were as readily infected as any other stage, it is suggested that they may be more practical for use in xenodiagnosis. Larger numbers can

be utilized and they would not be so repugnant to the person being bitten as larger adults and nymphs. Species of bugs used for xenodiagnosis should be from the same area as the T. cruzi strains.

On an individual basis, T. s. texana and T. gerstaeckeri would be as efficient as vectors of T. cruzi as R. prolixus, if they were not reluctant to crawl on the host and defecated to a greater extent while feeding. These two factors alone could account for the apparent paucity of human cases of American trypanosomiasis in Texas. It is not considered likely that American trypanosomiasis will become endemic in humans in the southwest. In addition to the factors mentioned, humans in this area are not subjected to repeated attacks by large numbers of bugs as those in more primitive areas of South and Central America.

A few individuals of each species fed, on outdated human blood, through natural and artificial membranes. A small percent of these became infected with T. cruzi organisms that had been added to the blood. Wood rat skins and Baudruche membranes gave the best results. Adults fed better than nymphs and R. prolixus fed better than the other two species.

In a two year period 142 wood rat dwellings were examined for bugs 85 (68.5%) contained a total of 213 nymphs and 16 adults of T. s. texana and T. gerstaeckeri. The mean number of bugs per infested dwelling was 2.5 with a range of 1-22. Bugs were collected during all seasons of the year and were occasionally found several hundred feet from the nest of any known host. The condition of most bugs collected indicated that they had not fed for some time. Seventy-nine (30.1%) of the 229 bugs collected were positive for T. cruzi-

like organisms. A higher percent of adult bugs were infected than nymphs.

During 1966 and 1967, 698 adult T. gerstaeckeri were collected in blacklight traps. The first bugs were collected in April and the last in September. Males appeared in the spring approximately two weeks before the females. The largest flights occurred in May, June, and July when the average minimum-maximum temperature was within 66.5 - 95 F. In 15 trap nights, 89.1 percent of the bugs were collected between 12:00 - 7:00 A.M. when the average minimum-maximum temperature was 76.4 - 84 F. It is concluded that optimum flight conditions exist when the temperature is within 75 - 85 F. In summer this frequently does not occur until the hours between 12:00 - 7:00 A.M. There was no observable correlation between light intensity and catch of the bugs.

Factors other than temperature must stimulate flight in Triatoma. Only two of 698 bugs collected in traps appeared to have fed recently and the mean survival time of female bugs was 13 days without feeding. Not all female bugs collected laid fertile eggs. It is concluded that female bugs require a blood meal within a relatively short period of time after flight to survive, and not all females are fertilized prior to flight.

Of 698 bugs examined, 544 (77.9%) were infected with T. cruzi-like organisms. Infection rates and parasitemias were higher in adult bugs collected in traps located where armadillos were most common than where wood rats were the predominant host animal. The high infection rate in trap collected bugs as compared with nymphs and adults collected in wood rat dwellings is difficult to explain. Unfortunately, collections were not made from arma-

dillo dens. It is felt that if extensive collections are made from armadillo dens the infection rate in nymphs will be higher than in those bugs collected from wood rat dwellings. The armadillo could be the primary host of T. gerstaeckeri in Texas and this bug may eventually be found throughout the same range as this mammal.

Only 13 specimens of T. s. texana were collected in light traps. Apparently this species is not attracted to black light as frequently as T. gerstaeckeri, or they may not fly in great numbers.

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APPENDIX A

TABLE I

FECUNDITY AND LONGEVITY OF ADULT TRIATOMINAE
AT 65-82F and 20-65% R.H.

Species and Pair	No. Eggs	No. Hatch	% Hatch	Longevity in Days	
				Male	Female
T. s. texana					
A	1066	521	49	483	813
B	697	495	71	623	670
C	340	236	69	425	423
D	809	497	60	582	665
E	651	525	80	521	375
Average	712.6	454.8	65.8	527.4	589.2
T. gerstaeckeri					
F	678	460	68	299	381
G	836	618	75	417	369
H	965	664	68	245	275
I	505	363	72	263	221
J	393	286	73	205	180
Average	675.4	478.2	71.2	285.4	285.0
R. prolixus					
K	517	368	70	312	612
L	497	378	76	148	219
M	151	136	90	219	146
N	651	534	82	279	261
O	351	302	86	378	192
Average	433.5	343.6	80.8	267.2	286.0

TABLE II

FECUNDITY AND LONGEVITY OF ADULT TRIATOMINAE
AT 80F and 65% R.H.

Species and Pair	No. Eggs	No. Hatch	% Hatch	Longevity in Days	
				Male	Female
T. s. texana					
AA	297	239	81	517	412
BB	742	586	79	692	614
CC	468	398	83	413	298
DD	949	739	80	619	576
EE	639	549	86	787	682
Average	619.0	502.2	81.8	625.6	516.4
T. gerstaeckeri					
FF	1069	887	83	298	369
GG	262	197	82	314	211
HH	742	527	71	368	302
I I	578	497	86	342	269
J J	612	496	81	263	412
Average	652.6	520.8	80.6	317.4	312.6
R. prolixus					
KK	702	611	87	297	247
LL	362	333	92	312	241
MM	412	330	80	249	398
NN	519	431	83	459	416
OO	469	399	85	279	187
Average	492.8	420.8	85.4	319.2	297.8

TABLE III

MAXIMUM NUMBER OF EGGS LAID AFTER ONE COPULATION

Species and Bug	No. Eggs	No. Hatch	% Hatch	Longevity in Days
T. s. texana				
A1	666	373	56	542
B1	572	361	63	419
C1	197	139	70	226
D1	819	478	58	612
E1	467	316	68	396
Average	544.2	333.4	63.0	439.0
T. gerstaeckeri				
F1	397	289	73	198
G1	712	432	61	401
H1	516	408	79	212
I 1	431	268	62	267
J 1	479	246	51	319
Average	507.0	308.6	65.2	279.4
R. prolixus				
K1	316	236	75	186
L1	519	362	69	314
M1	420	265	70	239
N1	469	291	61	469
O1	398	271	68	419
Average	424.4	230.8	68.6	325.4

TABLE IV

REPRODUCTIVE CAPACITY AND LONGEVITY OF UNFED MALE AND FEMALE TRIATOMINAE

Species and Pair	No. Eggs	No. Hatch	% Hatch	Longevity in Days	
				Male	Female
T. s. texana					
A2	4	1	25.0	42	31
B2	3	0	0	31	17
C2	2	0	0	27	14
D2	13	6	46.1	16	48
E2	9	5	55.5	24	25
Average	6.2	2.4	38.6	28.0	27.4
T. gerstaeckeri					
F2	2	0	0	23	14
G2	0	0	0	36	9
H2	14	4	28.5	22	37
I 2	9	3	33.3	41	16
J 2	6	1	16.6	7	24
Average	6.2	1.6	25.8	25.8	20.0
R. prolixus					
K2	3	1	33.3	21	17
L2	0	0	0	17	9
M2	4	0	0	19	28
N2	11	3	27.2	32	11
O2	8	2	24.4	29	20
Average	5.2	1.2	23.0	23.6	17.0

TABLE V

DEVELOPMENTAL PERIOD, IN DAYS, AT DIFFERENT TEMPERATURES

Species and Stage	65-86 F			80 F		
	Min	Max	Mean	Min	Max	Mean
T. s. texana						
Egg	22	26	23.6	21	23	22.8
1st	45	77	53.4	23	36	28.6
2nd	27	60	46.1	47	41	37.8
3rd	39	131	72.8	51	75	61.8
4th	81	197	126.6	56	109	84.8
5th	60	72	65.3	69	111	85.3
Total			387.8			322.1
T. gerstaeckeri						
Egg	25	28	26.4	24	27	17.1
1st	38	61	53.2	21	29	14.8
2nd	22	31	27.4	19	25	15.7
3rd	38	94	68.2	27	33	17.3
4th	78	168	107.1	47	132	71.3
5th	63	91	79.6	59	96	77.7
Total			361.9			213.9
R. prolixus						
Egg	18	22	20.7	15	19	18.1
1st	11	21	16.7	12	20	14.8
2nd	14	24	20.2	13	23	15.7
3rd	18	20	19.7	15	25	17.3
4th	15	27	21.8	11	26	20.5
5th	23	33	27.0	21	31	25.7
Total			126.1			112.1

TABLE VI

FEEDING AND MOLTING OF FIRST INSTAR NYMPHS FED ON VARIOUS HOST ANIMALS

Host	T. s. texana		T. gerstaeckeri		R. prolixus	
	Fed(%)	Molt(%)	Fed(%)	Molt(%)	Fed(%)	Molt(%)
Baby White Mouse	15(37.5)	1(.06)	25(62.5)	16(64.0)	30(75.0)	27(90.0)
Adult White Mouse	26(65.0)	1(.04)	31(77.5)	21(67.7)	35(87.5)	32(90.0)
Adult Wood Rat	25(63.0)	0	30(75.0)	18(60.0)	39(97.5)	36(90.2)
Toad (Bufo sp.)	7(17.5)	0	22(55.0)	12(54.9)	14(33.5)	11(78.5)
Skink (Eumeces sp.)	5(12.5)	0	18(45.0)	7(38.8)	27(67.5)	14(51.8)
Snake (Thamnophis sp.)	5(12.5)	0	21(52.5)	5(23.8)	18(45.0)	2(11.1)
Baby Chick	14(35.0)	0	24(60.0)	11(45.8)	16(40.0	13(81.2)
Ground Squirrel (Spermophilus sp.)	24(60.0)	0	31(77.5)	26(83.8)	33(82.5)	29(87.8)
Armadillo (Dasypus sp.)	22(55.0)	0	35(87.5)	27(77.1)	32(80.0)	27(84.3)
Human	11(27.5)	0	27(67.5)	16(59.2)	40(100)	38(95.0)
Engorged Bugs	4(10.0)	0	3(7.5)	0	1(.02)	0

TABLE VII

DEFECATION AND FEEDING TIMES OF TRIATOMINAE

Species and Stage	No. Bugs	No. & (%) Defecating Within 2 min. after Feeding	Mean Feeding Time in Minutes
T. s. texana			
1st	10	0 (0)	18
2nd	12	1 (8.3)	23
3rd	15	2 (13.3)	29
4th	15	4 (26.6)	25
5th	15	3 (20.0)	31
Adult Male	16	0 (0)	22
Adult Female	53	13 (24.5)	27
T. gerstaeckeri			
1st	15	1 (6.6)	18
2nd	10	1 (10.0)	19
3rd	12	2 (16.6)	31
4th	16	3 (18.7)	33
5th	20	6 (30.0)	39
Adult Male	19	0 (0)	20
Adult Female	68	18 (26.4)	27
R. prolixus			
1st	20	15 (75.0)	7
2nd	20	14 (56.0)	10
3rd	16	13 (81.2)	16
4th	18	16 (88.8)	17
5th	25	20 (80.0)	19
Adult Male	30	17 (56.6)	12
Adult Female	40	31 (77.5)	13

TABLE VIII

MEAN WEIGHT AND AMOUNT OF BLOOD INGESTED BY TRIATOMINAE

Species	Stage	No. Bugs	Wt. Before Feed In mg	Wt. After Feed In mg	Amt. Blood Taken In mg	% Gain
T. s. texana	1st	10	.47	1.62	1.25	265.95
	2nd	10	1.89	6.10	4.21	222.75
	3rd	10	5.64	17.28	11.64	206.38
	4th	10	24.26	70.45	46.19	190.39
	5th	10	62.79	192.47	129.68	206.52
	Adult Male	10	76.21	128.83	52.61	69.03
	Adult Female	10	88.35	167.71	79.36	89.82
T. gerstaeckeri	1st	10	.75	4.76	4.01	534.66
	2nd	10	2.36	9.81	7.45	315.67
	3rd	10	9.76	37.22	27.46	281.35
	4th	10	33.17	94.55	61.38	185.04
	5th	10	89.51	275.72	186.21	208.03
	Adult Male	10	152.31	284.90	132.59	87.05
	Adult Female	10	183.80	401.48	217.68	118.43
R. prolixus	1st	10	.45	3.66	3.21	713.33
	2nd	10	1.97	15.78	13.81	701.01
	3rd	10	6.23	54.96	48.73	782.18
	4th	10	21.96	148.99	127.03	578.46
	5th	10	45.22	282.93	237.71	525.67
	Adult Male	10	89.67	256.45	129.67	144.60
	Adult Female	10	118.31	305.50	187.19	158.21

TABLE IX

VIRULENCE OF TWO STRAINS OF T. CRUZI TO LABORATORY ANIMALS

Strain and Route of Inoculation	No. Animals	No. Trials	No. Infected	Tryps/Hi Dry Field	Deaths
Lackland					
Oral	5	2	7	10/100	0
Intramuscular	5	2	8	14/100	0
Brazilian					
Oral	5	2	6	6/100	0
Intramuscular	5	2	9	27/100	1

TABLE X

INFECTION OF EXPERIMENTAL ANIMALS BY VARIOUS ROUTES OF INOCULATION

Route of Inoculation	Animals	Trials	Infected	Tryps/Hi Dry Field	Deaths
Bite of Infected Bug	15	1	0	0	0
Oral Contamination	3	2	5	50/100	0
Unbroken Skin	3	2	1	2/100	0
Eye Contamination	3	2	2	2/100	1
Ingestion of Infected Bugs	10	1	7	200/100	1
Intramuscular	5	2	8	700/100	1
Intraperitoneal	5	2	7	400/100	2
Direct to Stomach	5	1	2	0 +by blood culture	0

TABLE XI

THE SUSCEPTIBILITY OF TRIATOMINAE TO TWO STRAINS OF T. CRUZI

Species	Stage	Brazilian Strain			Lackland Strain		
		No. Fed	No. Inf.	% Inf.	No. Fed	No. Inf.	% Inf.
T. s. texana	1st	19	13	68.4	22	18	81.8
	2nd	14	10	71.4	12	9	75.0
	3rd	15	13	86.6	13	10	76.9
	4th	12	8	66.6	9	7	77.7
	5th	10	7	70.0	7	6	85.7
	Adult Male	12	10	58.3	10	8	80.0
	Adult Female	14	10	71.4	11	10	90.9
T. gerstaeckeri	1st	97	71	73.1	110	91	82.7
	2nd	50	38	76.0	48	34	70.8
	3rd	26	19	73.0	25	18	72.0
	4th	19	15	78.9	16	13	81.2
	5th	15	13	86.6	19	17	89.4
	Adult Male	20	14	70.0	22	17	77.2
	Adult Female	20	16	80.0	24	21	87.5
R. prolixus	1st	50	36	72.0	50	36	72.0
	2nd	37	29	78.3	35	25	71.4
	3rd	26	19	73.0	19	15	78.9
	4th	18	13	72.2	22	15	68.1
	5th	16	13	81.2	18	13	72.2
	Adult Male	15	11	73.3	10	7	70.0
	Adult Female	15	12	80.0	12	9	75.0

TABLE XII

SUSCEPTIBILITY OF TRIATOMINAE FED ON INFECTED ANIMALS WITH NO OBSERVABLE CIRCULATING PARASITEMIA

Species	Stage	No. Fed	No. Inf.	% Inf.
T. s. texana	1st	14	9	64.2
	2nd	9	6	66.6
	3rd	13	9	69.2
	4th	10	7	70.0
	5th	11	8	72.7
	Adult Male	8	5	62.8
	Adult Female	10	7	70.0
Mean				67.9
T. gerstaeckeri	1st	42	32	78.5
	2nd	30	19	63.3
	3rd	19	12	63.1
	4th	15	11	73.3
	5th	10	7	70.0
	Adult Male	12	8	66.6
	Adult Female	15	11	73.3
Mean				69.7
R. prolixus	1st	50	27	54.0
	2nd	20	11	57.5
	3rd	20	13	65.0
	4th	20	11	55.0
	5th	15	10	66.6
	Adult Male	15	9	60.0
	Adult Female	19	13	68.4
Mean				60.9

TABLE XIII

RELATIVE NUMBER OF TRYPANOSOMES (PER 100 FIELDS) PASSED IN ADULT BUG FECES

Days Post Infection	T. s. texana					T. gerstaeckeri					R. prolixus				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	x	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	x	x	x	0	0	0	x	0	0	0	0	0
4	x	0	x	x	x	x	x	x	x	x	0	x	0	0	0
5	0	x	x	x	x	x	x	x	x	0	x	0	0	x	0
6	x	x	0	x	x	0	x	x	x	x	1	x	2	0	x
7	x	x	x	x	x	x	0	x	x	x	x	x	x	0	x
Fed on Uninfected Host															
8	10	1	1	3	70	9	17	14	100	40	16	7	44	0	20
9	2	1	x	5	40	7	4	20	70	18	5	3	20	0	15
10	0	x	x	x	45	x	x	7	55	14	19	6	17	0	12
11	x	1	1	1	x	4	9	x	x	x	x	2	6	x	10
12	0	x	x	x	x	x	x	x	17	x	x	3	10	0	15
13	x	x	x	1	5	1	x	1	x	x	4	5	x	x	x
14	x	x	x	x	x	x	3	x	x	1	x	x	9	0	7
Fed on Uninfected Host															
15	21	50	5	30	300	20	550	100	600	150	25	50	100	0	45
16	4	x	x	100	150	1	20	50	250	50	20	60	5	0	30
17	x	10	1	25	x	x	x	12	25	18	1	x	12	0	7
18	1	x	x	x	9	7	14	4	x	1	14	25	1	x	x
19	x	2	x	x	x	x	x	x	1	x	x	14	x	0	0
20	x	x	x	9	25	1	x	7	x	x	9	x	1	0	x
21	x	x	x	x	x	x	6	x	3	x	x	3	x	x	4

x - No defecation

TABLE XIV

INFECTION AND FEEDING OF FIRST INSTAR NYMPHS AND ADULT TRIATOMINAE THROUGH MEMBRANES

Membrane	Nymphs				Adults			
	No. Bugs	No. Fed	% Fed	No. Inf.	No. Bugs	No. Fed	% Fed	No. Inf.
				T. s. texana				
Wood Rat Skin	15	8	53.3	1	9	5	55.5	1
Naturalamb*	10	0	0	0	7	0	0	0
Baudruche**	10	2	20.0	0	5	1	20.0	0
Parafilm "M"***	12	0	0	0	5	0	0	0
				T. gerstaeckeri				
Wood Rat Skin	30	18	60.0	3	12	8	66.6	2
Naturalamb	45	6	13.3	0	7	2	28.5	0
Baudruche	28	7	28.5	1	10	3	30.0	1
Parafilm "M"	19	2	10.5	0	9	1	22.2	0
				R. prolixus				
Wood Rat Skin	25	18	72.0	6	12	9	75.0	2
Naturalamb	16	5	31.2	0	15	5	33.3	0
Baudruche	20	14	70.0	1	14	8	57.1	1
Parafilm "M"	30	7	23.3	0	10	3	30.0	0

*-Prophylactic skins, Youngs Rubber Corp., New York, N.Y.
**-Bovine Intestine, Long and Long Co., Belleville, N.J.
***-American Can Co., Menosha, Wis.

TABLE XV

DEVELOPMENTAL PERIOD, IN DAYS, UNDER FIELD CONDITIONS

Stage	T. s. texana		T. gerstaeckeri	
	Min	Max	Min	Max
Egg	14	41	12	42
1st	40	151	22	122
2nd	37	45	26	42
3rd	41	81	27	36
4th	233	286	41	47
5th	165	289+	178	237

TABLE XVI

COMPARATIVE FECUNDITY AND LONGEVITY OF TRIATOMA UNDER FIELD CONDITIONS

Species	No. Eggs	No. Hatch	% Hatch	Longevity in Days	
				Male	Female
T. s. texana					
A	167	96	57.4	680	585
B	465	279	60.0	329	476
C	242	152	62.8	419	388
T. gerstaeckeri					
D	529	307	58.0	392	210
E	660	401	60.7	289	377
F	803	485	60.5	312	379

TABLE XVII

NUMBER AND VIABILITY OF TRIATOMA EGGS UNDER FIELD CONDITIONS

Month (1966)	T. s. texana			Average Temperature F		T. gerstaeckeri		
	No. Eggs	No. Hatch	% Hatch	Max	Min	No. Eggs	No. Hatch	% Hatch
March	5	3	60.0	73.8	53.7	10	7	70.0
April	43	29	67.4	80.0	61.7	114	89	78.0
May	52	45	86.5	84.7	70.9	151	138	91.3
June	131	93	70.9	89.5	71.8	188	128	68.0
July	82	36	45.0	93.5	73.1	88	37	45.4
Aug	70	26	37.1	92.6	72.9	87	29	33.3
Sept	63	42	66.6	86.2	71.1	63	42	66.6
Oct	19	5	26.3	75.2	56.8	51	13	25.4
Nov	0	0	0	69.8	55.4	45	2	4.4
Dec	0	0	0	63.4	48.3	6	0	0
Total	465	279	60.0			803	485	60.3

TABLE XVIII

SURVIVAL OF TRIATOMA UNDER FIELD CONDITIONS WITH ONE FEEDING

Species and Stage	No. Bugs	Survival in Days		
		Max	Min	Mean
T. s. texana				
1st	10	156	12	118
2nd	10	196	48	132
3rd	8	198	44	141
4th	7	181	97	152
5th	5	385+	119	168
Adult Male	4	238	92	126
Adult Female	5	195	78	119
T. gerstaeckeri				
1st	10	149	33	119
2nd	10	270	97	123
3rd	10	225	81	148
4th	8	189	49	151
5th	6	192	131	169
Adult Male	5	186	62	158
Adult Female	5	219	98	162

TABLE XIX

COMPARISON OF ADULT T. GERSTAECKERI COLLECTED IN LIGHT TRAPS AT TWO DIFFERENT SITES

Month (1967)	Lackland AFB				Southwest Bexas County			
	No. Bugs		No. (%) Infected		No. Bugs		No. (%) Infected	
	♀	♀	♀	♀	♀	♀	♀	♀
April	9	0	2(22.2)	0	0	0	0	0
May	35	12	28(80.0)	7(58.3)	40	19	34(85.0)	15(78.9)
June	27	56	19(70.3)	40(71.3)	70	47	58(82.8)	36(76.5)
July	3	9	3(100)	5(55.5)	85	29	75(88.2)	23(79.3)
Aug	0	5	0	5(100)	39	13	33(84.6)	3(23.0)
Sept	0	1	0	1(100)	10	8	6(60.0)	8(62.5)
Total	74	83	52(70.2)	58(69.8)	244	116	206(84.4)	85(73.2)

TABLE XX

COMPARISON OF ADULT T. GERSTAECKERI COLLECTED DURING SUCCESSIVE YEARS IN LIGHT TRAPS LOCATED IN THE SAME AREA

Month	1966				1967			
	No. Bugs		No. (%) Infected		No. Bugs		No. (%) Infected	
	♀	♀	♀	♀	♀	♀	♀	♀
May	No Traps				40	19	34 (85.0)	15 (78.9)
June	36	20	28 (77.7)	16 (80.0)	70	47	58 (82.8)	36 (76.5)
July	44	12	36 (81.8)	9 (75.0)	85	29	75 (88.2)	23 (79.3)
August	51	4	44 (86.2)	3 (75.0)	39	13	33 (84.6)	3 (23.0)
September	12	2	7 (58.3)	0	10	8	6 (60.0)	8 (62.5)
Total	143	38	115 (73.4)	28 (73.6)	244	116	206 (84.4)	85 (73.4)

TABLE XXI

TEMPERATURE AND LIGHT TRAP COLLECTION CORRELATIONS FOR T. GERSTAECKERI ON LACKLAND AFB

Month (1967)	Average Temperatures F Max	Min	Total No. Bugs Collected
April	86.6	67.9	9
May	88.7	66.5	47
June	95.8	74.3	83
July	94.9	74.3	12
Aug	91.8	74.6	5
Sept	83.3	68.6	1

TABLE XXII

TIME AND TEMPERATURE CORRELATIONS FOR T. GERSTAECKERI COLLECTED IN LIGHT TRAPS

Time Period (1967)	Trap Nights	Average Temperature F Max	Min	Total No. Bugs Collected
19 June - 21 July	15			
6:00-12:00 p.m.		96.9	83.4	5
12:00-7:00 a.m.		84.0	76.4	41

APPENDIX B

Figure 1. Rearing Container for Bugs

Figure 2. Rat Confined in Feeding Chamber

Figure 3. Cage Holder with Restrained Rat and Feeding Bug

Figure 4. Bug Suspended on Glass Rod

Figure 5. Suspended Bug Feeding on Restrained Rat

Figure 6. Bugs Suspended Over Defecation Chambers

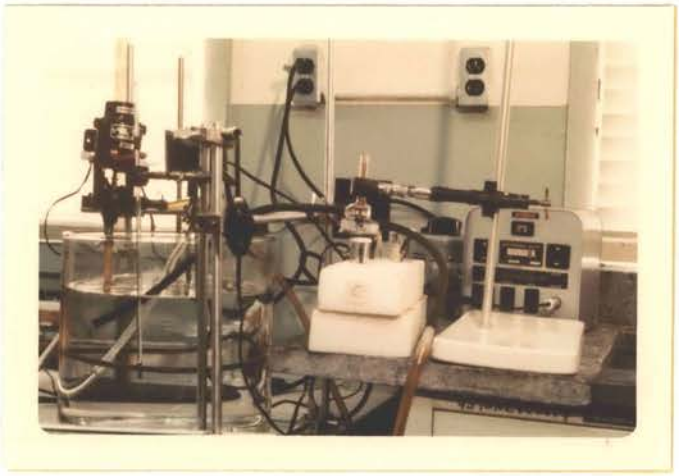

Figure 7. Membrane-Feeding Apparatus

Figure 8. Bug Positioned Under Membrane

Figure 9. Outdoor Cage

Figure 10. Simulated Wood Rat Nest in Outdoor Cage

Figure 11. Clump of Cactus Containing Several Wood Rat Nests

Figure 12. Wood Rat Nest at Base of Cactus Plant

Figure 13. Standard Blacklight Trap Used in Survey

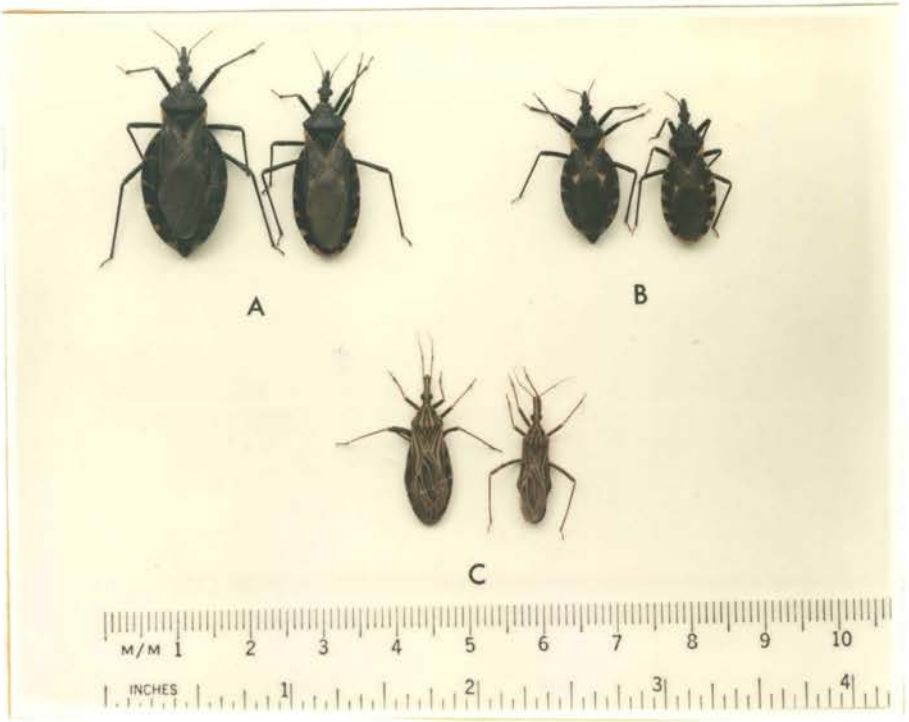

Figure 14. Adults of T. gerstaeckeri (A), T. s. texana (B), and R. prolixus (C). Females on the left.

Figure 15. First Instar Nymphs of T. gerstaeckeri Feeding on Emerging Adult of R. prolixus

VITA

WARREN FLOYD PIPPIN

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE BIOLOGY AND VECTOR CAPABILITY OF TRIATOMA SANGUISUGA TEXANA USINGER AND TRIATOMA GERSTAECKERI (STAL) COMPARED WITH RHODNIUS PROLIXUS (STAL) (HEMIPTERA: TRIATOMINAE)

Major Field: Entomology

Biographical:

Personal Data: Born at Mulberry, Kansas, February 26, 1919, the son of Arnold G. and Nellie E. Pippin.

Education: Received the Bachelor of Science degree from the Colorado State University, with a major in Entomology, in May, 1941; received the Master of Science degree from the Oklahoma State University, with a major in Entomology, in May, 1958; completed requirements for the Doctor of Philosophy degree in May, 1968.

Experience: U.S. Army, 1942-46; U.S. Department of Agriculture, Agricultural Research Service, Plant Quarantine Division, 1946-52; from April, 1952 to present date, Medical Entomologist, U.S. Air Force.

Societies: Sigma Xi, Phi Sigma, Alpha Zeta, Beta Beta Beta, RESA, Entomological Society of America, and the American Mosquito Control Association.