A SURVEY OF CERTAIN WILD RODENTS FOR THE PRESENCE OF AMERICAN TRYPANOSOMIASIS IN OKLAHOMA

By

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IN OKLAHOMA

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PREFACE

Historically American trypanosomiasis has been regarded as a disease of humans found in the tropics and subtropics of the western hemisphere. The United States has always been excluded from consideration, although the disease occurs enzootically in many mammals, principally the wood rat, *Neotoma* spp., armadillo, *Dasypus* spp., and the opossum, *Didelphis* spp. .

While a member of the United States Air Force it was my privilege to be assigned to Oklahoma State University for a course of instruction leading to the degree of Master of Science. Because human cases of American trypanosomiasis had recently appeared in the United States and infections in rodents had been found within 100 miles of Oklahoma, an intensive study of possible American trypanosomiasis in rodents and arthropods was selected for a research problem. Subsequently the problem was resolved into a survey to determine if the disease was present in Oklahoma as an enzootic among certain wild rodents, principally the wood rats, cotton rats, and the white footed mice.

I am indebted to Drs. D. E. Howell and D. W. Twohy for guidance, suggestions and loan of equipment necessary to carry out the project. My special thanks are extended to W. F. Pippin, Captain USAF(MSC) a fellow student, for his assistance in the field and laboratory phases of the project. I am further indebted to the members of the thesis committee, Drs. R. R. Walton, D. E. Bryan, and Professor Q. B. Graves for their reviews of the manuscript. The advice and gracious loan of traps of Dr. A. M. Stebler and for the identification of rodent specimens by Dr. B. P. Glass are acknowledged. The kind permission to trap in the state parks and the many kindnesses extended by the superintendants of these public lands, both federal and state were greatly appreciated.

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CHAPTER I

INTRODUCTION

History of the Disease

Earlier works on American trypanosomiasis have not included the United States in the distribution of the malady among humans, for it was not until 1955 that Woody and Woody reported the first indigenous human case in the United States. This first case was followed by a second, likewise in the state of Texas. The records concerning this latter case are deposited with the Texas State Department of Health (Eads 1957).

The original description of *Trypanosoma cruzi* was prepared by Carlos Chagas in 1909 from organisms collected from the gut of *Panstrongylus megistus* (Burm.). The infected bugs were allowed to bite a monkey and the organism was later recovered from the animal. Chagas called this parasite *Schizotrypanosoma* in the mistaken belief that the transmission of the parasite was by the bite of the bug and that schizogeny occurred in the definitive host (Strong 1942). Brumpt in 1912 disproved the salivary transmission of the disease and demonstrated the parasite in the feces of the bug (Usinger 1944). Later, Chagas found what was apparently the same organism in the peripheral blood of a child. The child was suffering from an undiagnosed fever accompanied by anemia and a swelling of the lymph nodes. In 1916 Chagas published an account of 29 clinical cases observed over the years. All cases up to this point were from the state of Minas Geraes, Brazil. Furthermore, all cases were in infants or young children (Yorke 1937, Strong 1942). Chagas reported that one of the cardinal symptoms of the disease was goiter. This fact caused considerable confusion particularly where the disease was encountered elsewhere without the goitrous condition. It was later demonstrated that the trypanosomiasis was superimposed upon an endemic goiter common to the state of Minas Geraes (Yorke 1937).

Villela and Vichalo were called upon in 1923 to investigate conditions in a railroad camp in Minas Geraes relative to the incidence of American trypanosomiasis. They inoculated the blood of 19 suspected cases of American trypanosomiasis into guinea pigs, and obtained five positives (Yorke 1937).

Kofoid and McCulloch (1916) discovered a trypanosome in the cone nosed bugs (Hemiptera; Triatominae) in California. The trypanosome was not identified at that time. The first naturally infected mammal in the United States was reported by Wood (1934) in Neotoma fuscipes macrotis (Thomas), also in California.

Kofoid and Donat (1933), Packchanian (1939) and Wood and Wood (1941) have shown the trypanosomes of Trypanosoma cruzi spp. from California to Texas. All were naturally infected.

Woody and Woody's (1955) diagnosis of human trypanosomiasis was confirmed by Packchanian and also workers at the Tulane School of Tropical Medicine, New Orleans, La. It is of interest to note that both cases of the disease in the United States were discovered by accident during routine clinical examinations. In both cases the trypanosomes were discovered in central nervous system tissue. In the second case the trypanosomes were also found in the blood.

Both cases were children, the former a seven year old female and the latter a three week old male.

Geographical Distribution

For many years Minas Geraes was thought to be the endemic center of the disease. However, in 1919 cases were reported from both Venezuela and Peru. Although Minas Geraes remained the geographical center the disease has been found in more widely dispersed areas (Strong 1942).

The disease is now known to have a range extending from latitude 41° South to latitude 38° North (Yorke 1937, Wood and Wood 1941, Eads 1957).

Human cases have been recorded from the following countries: Brazil, Argentina, Venezuela, Peru, Bolivia, El Salvador, Uruguay, Guatemala, Panama, Mexico and the United States.

Various mammals have been found infected throughout the entire range of distribution. Within the United States, the states of California, Arizona, New Mexico and Texas have been shown thus far to have naturally infected hosts. Texas alone has had two recognized human cases to date.

Griffith (1948) and Kilpatrick (1953) gave accounts of the infestations of various households in Oklahoma City by triatomine bugs. Grundeman (1947) states that in the Manhattan, Kansas area these bugs are frequently found associated with wood rat nests and occasionally in the nests of the Texas cotton rat, Sigmodon hispidus texianus Audubon and Backman. Dr. Hopla, Medical Entomologist at the

University of Oklahoma (personal communication) further confirmed this for southwestern Kansas.

Incidence of the Disease

There exists a difference of opinion among authorities as to the prevalence of the disease. Strong (1942) states that the disease is not believed to be prevalent, but rather wide spread throughout its range. Yorke (1937) remarks that the total number of cases discovered up to 1937 was remarkably small. Hall (1953) cites the following incidence based upon the results of surveys using the xenodiagnosis and complement fixation tests: Chile, of 8142 individuals 17% were infected; Argentina, 23-42%; Bolivia, 6-31%; Brazil, 15-51%; Uruguay, 6%; and Venezuela, 27-46%; all in smaller group surveys than in Chile. These data point up the fact that more thorough research is needed throughout the Americas, particularly in the United States, to determine the prevalence of the disease.

In Texas of 859 specimens of triatomine bugs submitted for examination for trypanosomes of *T. cruzi*, 286 or 33.3% were found infected. *Triatoma gerstaeckeri* (Staal), *T. lecticularius* (Staal), *T. protracta* (Uhl.), *T. sanguisuga* (Lec.), *T. neotomae* Nieva and *T. rubida* (Uhl.) were all found to be infected with *T. cruzi*. The most heavily infected counties in Texas were: Ball, Dimmit, Hidalgo, Milam, and Uvalde (Sullivan et al. 1949).

Public Health Importance

Many workers have thought for years that American trypanosomiasis would be discovered in the United States as a natural infection in humans. The work of Packchanian (1940a) and Wood (1934) had shown this to be theoretically possible. Suitable vectors and reservoirs had been found, and it remained for Woody and Woody to discover the first case.

Kofoid and Whitaker (1936) and Mazzoti (1940) remark that the limiting factors against the spread of the infection in California are: (1) the reservoirs inhabit unpopulated areas, (2) not all vectors and rodents are infected, (3) a mild virulent strain of T. cruzi may exist in California, (4) the small numbers of trypanosomes in the peripheral blood of the reservoirs for a three week period, limiting the infection to the bug and the reservoir rodents, (5) limitations imposed by the means of infection, auto-inoculation.

In both human cases of the disease discovered to date the etiological agent was discovered by accident while routine clinical examinations were carried out. The physicians in the United States have little experience with this disease, thus it is entirely possible that many cases have been treated symptomatically and then released (Eads 1957). At this time there is no known cure for the disease (Usinger 1944, Craig and Faust 1951). The prognosis in the acute phase is serious. Even moderate infections have been known to persist for twelve years (Craig and Faust 1951).

A danger exists of the transfer of the disease by means of blood transfusions from the infected to the noninfected human (Eads 1957). It has been demonstrated experimentally in mice that the trypanosomes could be passed from the mother to the offspring by the mammae (Craig and Faust 1951). There also exists the possibility of new foci of the disease becoming established from one case in the human host being transferred to a suitable reservoir animal, such as Mus musculus (Linn.), Rattus rattus norvegicus (Berkenhaut) or Neotoma spp. .

More data are needed in the United States particularly, as well as in the rest of the Americas to establish what levels of infection actually exist in the human host and the animal reservoirs. Within the United States data are needed from the following states: Colorado, Oklahoma, Utah, Nevada, Arkansas and Louisiana to complete the distribution picture of the disease and the ecology associated with it. In the United States the reservoirs are for the most part sylvan or petrophyllic rodents. These are confined to principally rural or uninhabited regions, although domestic rodents have been incriminated.

Drs. D. E. Howell and C. Hopla (personal communications) have indicated that in past years many calls were received concerning reduviids entering homes throughout the state of Oklahoma and causing annoyance. Dr. Hassler, Director of the State of Oklahoma Public Health Laboratories has further confirmed this (personal communication). With the incrimination of domestic rodents, found naturally and artificially infected coupled with the fact that the peromyscine mice also will enter houses, another possible avenue of infection is thus established.

Vectors

The vectors of the disease belong to the family Reduviidae, sub family Triatominae of the order Hemiptera. In Table I will be found the vectors in South, Central and North America.

TABLE I

Vectors of *Trypanosoma cruzi* Found Naturally Infected in South, Central and North America.[1]

Cavernicola pilosa Barber	Brazil
Panstrongylus geniculatus (Latr.)	"
Triatoma rubrofasciata (DeGeer)	"
Triatoma chagasi Brumpt and Gomes	"
Triatoma infestans (Klug.)	Chile
Triatoma spinolai Porter	"
Triatoma dimidiata (Latr.)	Guatemala
Dipetalogaster maximus (Uhl.)	Mexico
Rhodnius prolixus (Staal)	"
Triatoma barberi Usinger	"
Triatoma dimidiata (Latr.)	"
Triatoma hegneri (Mazzoti)	"
Triatoma phyllosoma (Burm.)	"
Triatoma phyllosoma longipennis (Usinger)	"
Triatoma phyllosoma pallidipennis (Staal)	"
Triatoma phyllosoma picturata (Usinger)	"
Triatoma rubida (Uhl.)	"
Eratyrus cuspidiatus (Staal)	Panama
Panstrongylus geniculatus (Latr.)	"
Rhodnius pallescens (Barber)	"
Triatoma dimidiata (Latr.)	"
Triatoma dimidiata maculipennis (Staal)	El Salvador
Rhodnius prolixus (Staal)	"
Eratyrus cuspidiatus (Staal)	Venezuela
Eutriatoma nigromaculata (Staal)	"
Psammolestes arthuri Pinto	"
Panstrongylus megistus (Burm.)	"
Triatoma dimidiata (Latr.)	"
Rhodnius prolixus (Staal)	"
Triatoma gerstaeckeri (Staal)	United States
Triatoma lecticularius (Staal)	"
Triatoma recurva (Staal), (*longipes* Barber)	"
Triatoma protracta (Uhl.)	"
Triatoma protracta woodi Usinger	"
Triatoma rubida uhleri Neiva	"
Triatoma sanguisuga (Lec.)	"

[1] Compiled from Usinger 1944, Matheson 1950, and Craig 1951.

The vectors frequently live in the thatch and bamboo of native houses in the tropics, or in mammalian burrows, or trash piles in close proximity to human habitation. These insects show a decided preference for mammalian blood which is essential for their metabolism and growth. They are nocturnal in habit, flying during the early evening hours for the most part. The bite of the bugs in South American and Central American species characteristically causes little or no sensation during feeding or afterwards. Consequently few people that have been attacked could remember having been bitten (Strong 1942).

The ranges within the United States of the principal vectors are as follows: Triatoma sanguisuga (Lec.) and Triatoma lecticularius (Staal) are nearly coextensive within an area from the northern states of Mexico northward and eastward to Chesepeake Bay and south to the Gulf states, including all of Florida, with the northward extension of the range of Triatoma lecticularius being somewhat more restricted than that of Triatoma sanguisuga. Triatoma gerstaeckeri (Staal) is localized in its distribution to southeast Texas in the Brownsville area. Triatoma protracta (Uhl.) extends northward and westward from the Rio Grande valley and Baja California including Arizona, New Mexico, western Texas, Oklahoma Panhandle, Utah, Nevada, Colorado, California and parts of Oregon, and Wyoming. Triatoma longipes (Barber) is localized in southern Arizona (Usinger 1944).

Life Cycles of Triatomine Bugs

Eggs may be laid within nine days after the adult stage is reached. The usual time is from two to four weeks. Temperature of the environment plays a great part in the egg production. Growth is closely correlated with the availability of mammalian blood and achievement of a fully engorged condition (Wigglesworth 1934, Usinger 1944). The eggs of Rhodnius prolixus (Staal) and Triatoma lecticularius (Staal) are fastened to a substrate. Other species lay their eggs free, e.g. Triatoma gerstaeckeri (Staal), Triatoma longipes (Barber), and Triatoma rubida (Uhl.) (Usinger 1944).

Nymphs molt after the first feeding and must become fully engorged with the abdomen stretched to ovalness for the molting process to occur. No additional blood is taken until the molting process is completed. In feeding, the nymphs imbibe six to twelve times their body weight of blood, while the adults can only imbibe about three times their own weight (Usinger 1944).

The life cycle varies with the species and climatological factors. In South America the cycle may be completed in as short a time as six months, while the cycle of the species of more northern distribution may require up to a year and a half to two years for completion. In general, the adults appear in the spring, lay eggs in the summer and the nymphal stages develop throughout the winter months. The availability of mammalian blood and differences in climate causes variance from the one year cycle (Usinger 1944).

Animal Hosts

Table II lists the mammal hosts reported naturally infected with the disease.

Table III lists the rodents which Packchanian was able to inoculate and infect with Trypanosoma cruzi.

It is of interest to note that Chagas reported the domestic cat as the first reservoir animal in 1909 (Hall 1953).

TABLE II

Summary by Country of the Mammalian Hosts Naturally

Infected with Trypanosoma cruzi. 2/

Specific Name	Common Name of Host	Country
Dasypus novemcinctus (Linn.)	Armadillo	Brazil
Dasypus unicinctus (Linn.)	"	"
Felis sp.	Cat	"
Dasypus novemcinctus fenestratus Peters	Armadillo	Panama
Artibeus jamaicensis jamaicensis Leach	Bat	"
Desmodus rotundus murinus Osgood	"	"
Glossophaga soricina leachi (Gray)	"	"
Hemiderma perspicillatus aztecum (Sauss.)	"	"
Uroderma bilobata Peters	"	"
Canis sp.	Dog	"
Didelphis marsuplialis etensus Allen	Opossum	"
Sciurus gerradi morulus Bangs	Squirrel	"
Canis sp.	Dog	Guatemala
Dasypus novemcinctus mexicanus Peters	Armadillo	Mexico
Canis sp.	Dog	"
Neotoma (Hodymys) alleni (Merriam)	Wood rat	"
Antrozous pallidus pacificus Merriam	Bat	United States
Mus musculus (Linn.)	House mouse	"
Didelphis virginiana Kerr	Opossum	"
Neotoma lepida lepida Thomas	Wood rat	"
Neotoma micropus Baird	"	"

2/ Compiled from Usinger 1944, Matheson 1950, Craig 1948.

TABLE III

Artificial Infections of Trypanosoma cruzi in Mammals

Produced by Packchanian in the United States.

Specific Name	Common Name
Mus musculus (Linn.)	House mouse
Rattus rattus norvegicus (Berkenhaut)	Norway rat
Peromyscus eremicus eremicus Baird	White footed mouse
Peromyscus leucopus novaboracensis Fisher	"
Peromyscus polionotus polionotus Wagner	"
Neotoma fuscipes annectens Elliot	Wood rat

The association of vectors with the reservoirs in the United States was described by Wood (1934). The ecological niche contains a variety of organisms existing in the nests of wood rat. Besides the vector *Triatoma* spp., which was shown not to migrate from nest to nest,

> "....fleas, *Orchopeas caedens caedens* Jordan, and some *Hemiptera* spp., *Lepidoptera* spp., *Coleoptera* spp., mosquitoes, ixodine ticks, mites, lice and spiders were found in varying numbers in most of the nests."

The triatomine bugs complete the life cycle in the nest of the wood rat host. These vectors are intermittent feeders, dropping from the host when replete and hiding among the litter of the nest to digest the blood meal before molting. The adults are capable of flight and are nocturnal in habit. However, transmission of the disease probably occurs at the time of mating between the rats, since only one rat, with the exception of the young, occupies one nest. The male is driven off by the female after mating is completed (Wood 1934, Cahalane 1947). Wood (1934) showed that either all or none of the *Triatoma* taken from the nests were infected.

Description of the Disease

American trypanosomiasis in its early acute stages is characterized by fever and enlargement of the lymph nodes, liver, spleen and thyroid glands. The chronic phase shows visceral involvement with symptoms referable to the heart and endocrines (myxedema). The symptoms are protean in nature, the specific symptoms to some extent depend upon the organs involved. The disease can be fatal in either the acute or chronic phase, however, most human fatalities occur in infants under two years old. In most cases the chronic phase is never contracted by children less than two years of age. Most deaths result from a myocardial involvement (Merck Manual 1950, Craig and Faust 1951).

High fever is characteristic of the acute phase of the disease. The patient appears quite nervous, has psychic symptoms and may at times produce symptoms similar to encephalomyelitis. Death usually follows after the disease has run a course of two to four weeks, unless the chronic phase supervenes (Craig and Faust 1951).

The vectors in South America, Triatoma megista and Rhodnius prolixus, frequently bite at the outer canthi, or angles of the mouth. This feeding habit has earned these vectors in the South American countries the name "El Barbeiro", The Barber (Encyclopaedia Britannica 1955).

The initial lesion occurs at or near the locus of the bite. The parasite (T. cruzi) may gain entrance to the definitive host through the mucous membranes of the mouth or eye. Usually however, the infection is caused by auto-inoculation by scratching or abraiding the skin (Strong 1942). Packchanian (1940b) states that the parasite develops in the hind gut of the vector in large numbers as a crithidial (non-infective form). It changes into a metacyclic trypanosome (infective form) in the hind gut of the insect which is carried out of the alimentary tract when the gut is evacuated at the time of feeding. Wood (1951) and Keh (1956) definitely correlate the spread of the disease with the interval of time between feeding and defecation of the vectors. South American species defecate during, or shortly after feeding on the host while the North American forms usually defecate after they have left the host.

In the human host, the trypanosomes multiply by fission within the superficial lymph nodes, migrating thence to the spleen, liver, bone marrow, myocardium, thyroid and adrenal glands and the central nervous system. T. cruzi becomes intercellular as a leishmanial form producing proliferative and necrotic damage (Strong 1942).

Diagnosis depends upon the demonstration of trypanosomes in the peripheral blood by thick wet smears. The organism can only be found in the early stages by this method. As the disease progresses the organism may be identified in the spinal fluid, or fluid aspirations from the lymph nodes or other tissues (Strong 1942, Merck Manual 1950, Craig 1948). Xenodiagnosis may be employed if the disease is believed to have been contracted for a period of three weeks or longer (Craig and Faust 1951).

CHAPTER II

METHODS AND MATERIALS

Area Selected for Study

Blair and Hubbell (1938) classified the biota of Oklahoma into eleven districts: Ozark, Ouachita, Mississippi, Cherokee Prairie, Osage Savannah, Wichita, Mixed Grass Plains, Mesquite, Short Grass Plains, Mesa de Maya (known locally as Black Mesa) and Sand Dunes.

The polygon shaped area shown in (Figure 1) was selected originally as the territory to be surveyed in this study because it encompassed the majority of the biotic districts encountered in Oklahoma with two principal exceptions, e.g. the Mississippi district in the southeastern part of the state and the Short Grass Plains in the Panhandle. This original area was trapped extensively during the period from September 1956 to April 1957. In April of 1957 it was decided that check points should be established outside this area westward and southward towards areas of known infection in Texas. The plan was to use these new locations as checks against any infection found within the original territory. Still later it was decided that perhaps the entire state should be given consideration. Thus, trapping stations were set up in an arc extending from Black Mesa in the Panhandle southward along the southern counties of the state to the eastern border thence northwestward to Osage Hills State Park. This plan was started during August 1957. Since the results in the polygon shaped area had shown no trypanosomiasis endemic in the rodents trapped, this latter area was used as a check against the original stations. Trapping was terminated on September 30, 1957. In this survey of the state, eight of the eleven biotic districts of Blair and Hubbell were trapped. The exceptions were: Ozark, Mesquite, and Sand Dune districts.

Type of Traps Used

Fifteen "Havahart" 00 traps were purchased from the Allcock Manufacturing Company, Ossining, New York (Figure 2) and eighty five Blair traps (Figure 3) which were modified slightly, were utilized making a grand total of 100 traps.

Baits Employed

Dried raisins, sliced apples and a peanut-butter rolled oats mixture were tried as baits. The raisins worked well and were selected for their ease of use and transport in trapping.

Selection of Trapping Sites

Trapping sites were selected with regard to abundance of rodents, particularly *Neotoma spp.* . Public lands were selected to circumvent obtaining permission from many private land owners. The parks and refuges were selected purposely to represent the various types of biotic districts in this state.

The animals sought were: *Neotoma spp.*, *Peromyscus spp.* and *Sigmodon hispidus*. *Sigmodon hispidus* was trapped on the chance that this rodent might be a reservoir of the disease.

Traps were set in areas of known or suspected high incidence of

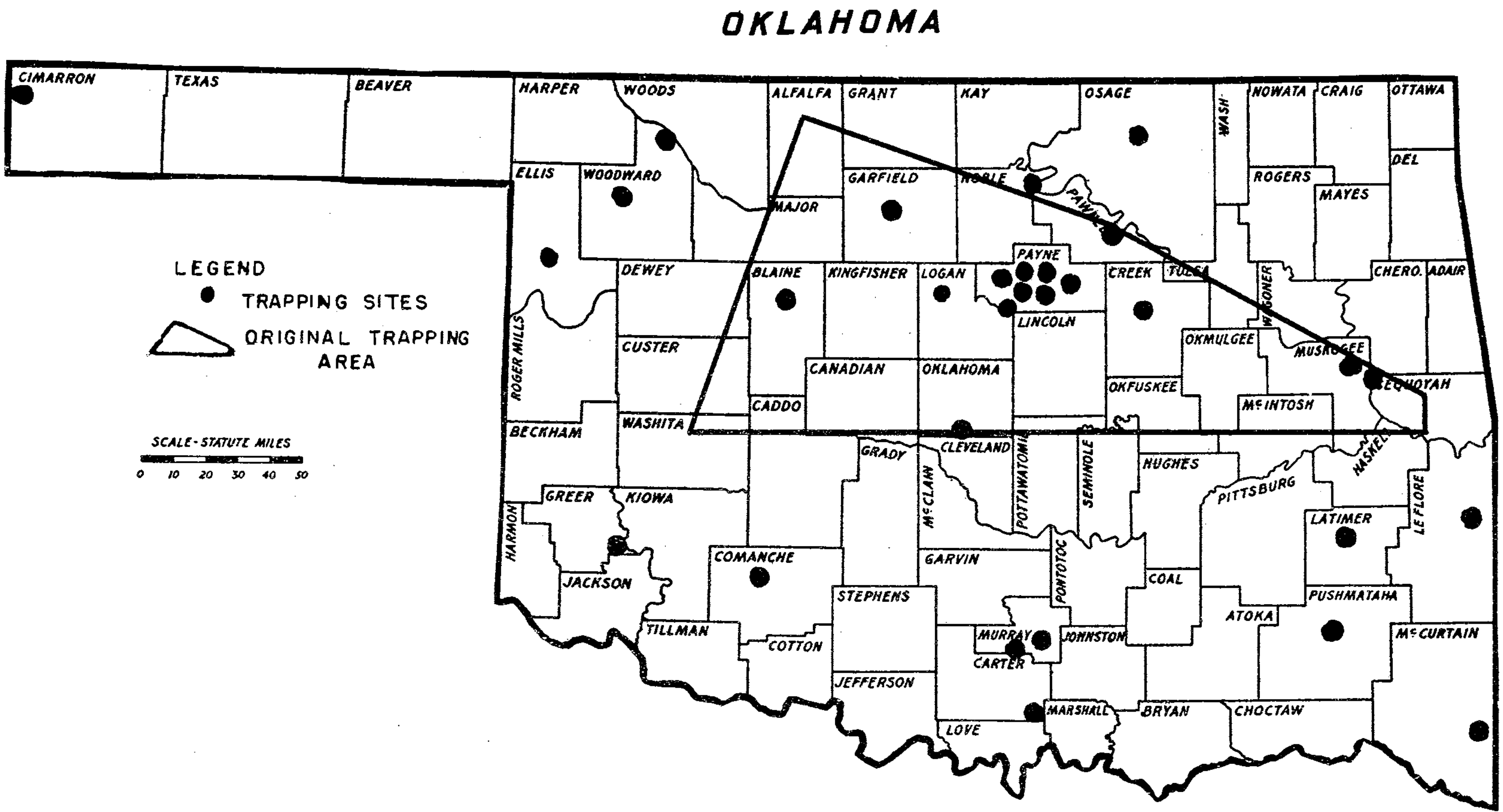

FIGURE - I. TRAPPING SITES USED IN OKLAHOMA BY COUNTIES FROM SEPT. 1956 TO SEPT. 1957.

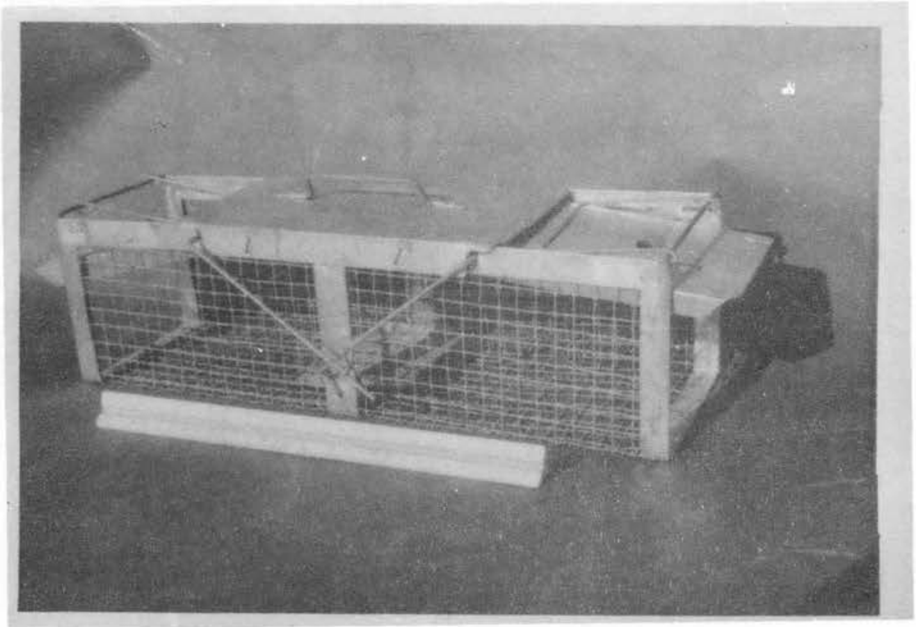

FIGURE-2. "HAVAHART" TRAP USED DURING THIS STUDY.

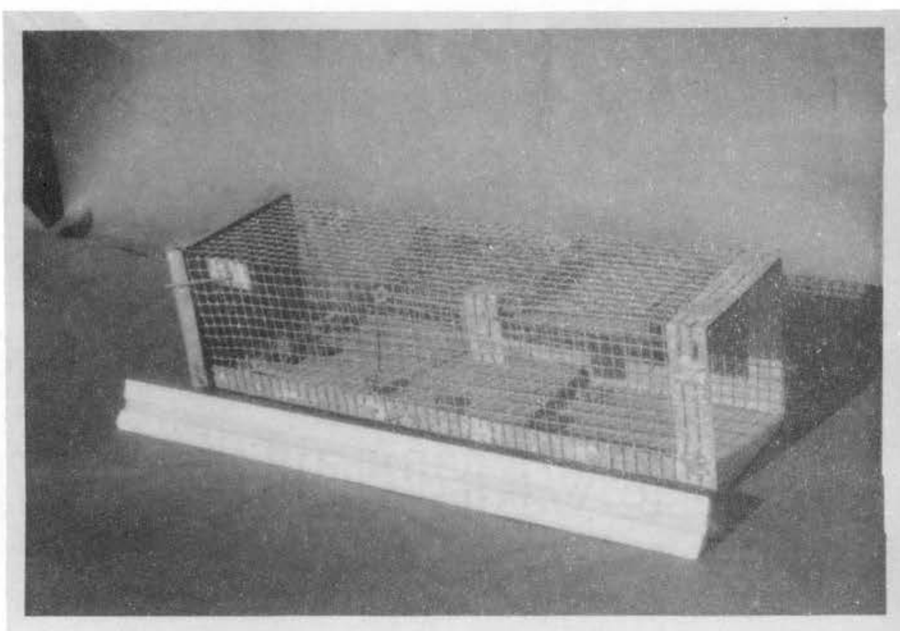

FIGURE-3. BLAIR TRAP USED DURING THIS STUDY.

rodent populations. Neotoma and Peromyscus are principally sylvan or petrophillic in habit. Sigmodon hispidus inhabits pastures and other grassy areas. The traps were baited liberally and set at the entrance to the burrows or in the runways.

A rough index to the rodent activity of Neotoma and Peromyscus in an area could be obtained from the fecal pellets or the urine stains on rocky outcroppings. There were no such indications for Sigmodon. However, some indication of activity of Sigmodon could be determined by the well beaten appearance of the grass in the active runways.

Prebaiting was not found necessary in the trapping of the above species. Where domestic rodents were being sought at the Stillwater Sewage Disposal Plant, prebaiting was practiced.

Traps were operated for two nights as a rule at each location.

Rodent Nests and Recovery of Triatomine Bugs

Wherever possible nest materials of the rodents were recovered by exposing the central portion of the nest, collecting the nesting material and sealing it in paper bags. Each bag was then returned to Stillwater. The contents were placed into Berlese funnels equipped with 300 watt bulbs and left for three hours. The bottom of the funnels were equipped with half pint Mason jars containing about an inch of water. The water trapped the arthropods escaping the heat generated by the bulbs in the funnels. The contents of the Mason jars were strained through bleached muslin, dried slightly under a 100 watt bulb and then examined for triatomine nymphs and other ectoparasites.

Blood and Tissue Examinations

Past work on the diagnosis of human and animal trypanosmiasis indicated that the examination of peripheral blood in early infections gave satisfactory results. However, since, the parasite disappears from the peripheral blood in about three weeks, this method would not be satisfactory for a survey. Thus the deep tissues must be examined for the various stages of the parasite.

Cardiac punctures were made on rats while the rat was anesthetized with chloroform or ether (no cardiac punctures were done on any of the mice). Five cubic centimeters of blood were withdrawn by means of a syringe. A drop of this blood was used to make a thin smear. The remaining blood was placed in a centrifuge tube with one drop of heparin to prevent clotting and centrifuged at 1500 rpm for five minutes. After centrifuging, one drop of the separated blood and one drop of the serum were admixed on a slide and drawn out into a thin smear. This technique was employed for the first 32 rats. Since the results were comparable to the thin smear technique this procedure was discontinued. Cardiac punctures were discontinued during August-September 1957. Dark field examination was used for a short while on thick drops of blood and then discontinued since the results were discouraging.

From March 1957-August 1957 a monochromatic stain was employed, Thedane T-3 (National Aniline Dye Corporation), which was developed for use with leishmanial, trypanosomal and other motile blood parasites. One drop of fresh blood was mixed with three loops (transfer loops) of Thedane T-3 for three to five seconds and then examined under high dry magnification (400 x). Any trypanosomes present should become immediately visible, since the stain haemolizes the blood cells leaving only the parasites. This method of examination was suggested by Dr. E. R. Schleicher, Department Head of Pathology, Mayo Clinic, Rochester, Minnesota.

The rats and mice were then killed with ether or chloroform. Pieces of tissue from the pectoral region of the heart, central lobe of the liver, and distal portion of the spleen were removed and smeared on slides.

The slides were then fixed in reagent grade methyl alcohol for three minutes, allowed to dry, and then placed in Giemsa stain (1:20 and buffered to pH 7.0) for twenty minutes, then rinsed with distilled water. Although Giemsa stain was retained, the dilution was reduced 1:40 and stained for three hours. The adequate staining time was found to be about six hours for best results. Thorough rinsing in distilled water was done to remove any residue of the stain from the slides.

Beginning in August 1957 the work was carried full time in the field. All specimens trapped were killed in the camp, rather than being returned to Stillwater as previously had been the case. The itinerary was such that a rigid time schedule had to be followed, thus the Thedane staining technique was no longer used. The trapped rodents were collected, killed and the tissues examined in the mornings. Due to the high summer temperatures encountered throughout the state all specimens had to be processed in the morning since high temperatures killed the animals. Dr. D. W. Twohy suggested that the tissue preparations of the rodents to be trapped during the remainder of field operations be crushed by placing the tissue slivers between two glass slides and crushing by applying pressure in a rotary motion. Before this was accomplished excess blood was allowed to drain off so as not to clutter the slide with excessive erythrocytes.

CHAPTER III

RESULTS

Trapping Operations

Trapping operations were started on September 30, 1956 and terminated September 30, 1957. A total of 151 rodents were trapped of which 133 were examined for trypanosomes. The remaining 18 animals either died from exposure during cold weather or high summer temperatures and due to deterioration could not be utilized for blood and tissue analysis. Table IV gives a summarization of the rodents trapped by species, subspecies and sex during the study.

TABLE IV

Total Numbers of Rodents Trapped and Examined by Species, Subspecies and Sex in Oklahoma from September 1956-September 1957.

Specific Name	Male	Female	Total
Neotoma floridana osagensis Blair	25	23	48
Neotoma floridana rubida Bangs	1	1	2
Neotoma micropus micropus Baird	4	7	11
Sigmodon hispidus texianus (Audubon and Backman)	13	9	22
Peromyscus boylii (Allen)	15	8	23
Peromyscus leucopus leucopus (Rafinesque)	5	6	11
Peromyscus maniculatus maniculatus (Hoy and Kennicott)	10	5	15
Peromyscus truei truei (Schufeldt)	1	0	1
Totals	74	59	133

Table V gives the location, the number of rodents trapped, and the number of trap nights in each area. One trap operated one night is considered to be one trap night.

In the spring of 1957 rodent populations diminished sharply. It was entirely possible that an epizootic or the heavy rains or combinations of the above factors were responsible. This reduction was particularly noticeable in cotton rat populations around Stillwater at the Airport and at Lake Carl Blackwell which had an abundance of the rodents in the early spring. The population did not begin to build up again until fall.

During the summer of 1957 Peromyscus boylii was the only species obtained in the latter part of August in an area extending from Lake Murray State Park through the Kiamichi Mountains to Robber's Cave State Park. The one specimen of Peromyscus truei truei was obtained at Black Mesa in Cimarron County. The two specimens of Neotoma floridana rubida were obtained from Beaver's Bend State Park in McCurtain County.

Rodent Nests and Recovery of Reduviids

A total of twenty rodent nests were collected, of which four were Sigmodon hispidus and the remainder were Neotoma spp. . No reduviids were recovered, although whip scorpions, fleas, (Orchopeas sp.) and dermestids and some mites were recovered from the nesting material after placing it in the Berlese funnel.

In January of 1957 members of the Oklahoma Pest Control Association were requested at their annual meeting at Oklahoma State University to send any triatomine bugs found during their state wide operations to the author for examination for trypanosomes. No specimens were received and subsequent communications have shown that few reduviids were encountered during the year.

TABLE V

Traps Sites, Number of Trap Nights, Number of Rodents Trapped and Successful Trapping Percentage Obtained, in Oklahoma from September 1956-September 1957.

County	Location	Total Trap Nights	Number of Rodents Trapped
Blaine	Roman Nose State Park	123	7
Carter	Lake Murray State Park	58	2
Comanche	Wichita National Refuge	70	7
Cimarron	Black Mesa	50	2
Creek	Heyburn Lake State Park	44	0
Ellis	Arnett	14	0
Garfield	Vance Air Force Base	100	0
Greer	Quartz Mountains State Park	78	3
Latimer	Robber's Cave State Park	24	1
LeFlore	Lake Wister State Park	53	10
Logan	LeBron Township	22	5
McCurtain	Beaver's Bend State Park	31	3
Murray	Turner Falls, Davis, City Park	25	0
"	Platt National Park, Sulphur	15	0
Muskogee	Greenleaf Lake State Park	8	1
Oklahoma	Tinker Air Force Base	44	0
Osage	Osage Hills State Park	45	4
Pawnee	Cleveland	75	8
"	Redrock	27	5
Payne	Coyle	28	2
"	Stillwater Airport	84	16
"	Stillwater Sewage Plant	30	0
"	Farm east of Stillwater	34	6
"	Lake Carl Blackwell	194	21
"	Stillwater, City Dump	12	1
Pushmataha	Clayton Lake State Park	80	8
Sequoyah	Tenkiller Lake State Park	46	24
Woodward	Boiling Springs State Park	93	13
"	Alabaster Caverns	19	2
	Totals	1525	151

Successful trapping percentage $\frac{151}{1525} = 9.90\%$

Only three triatomine bugs were obtained from other sources, all of which came from the Stillwater area. All of these specimens were *Triatoma sanguisuga*, two of which were received alive. These two bugs were examined for the presence of trypanosomes or crithidial forms and none were found. Two of these bugs were obtained from the interiors of homes and the other was obtained from a window screen near a porch light.

Blood and Tissue Examinations

All slides were negative for either leishmania or trypanosome forms of *Trypanosoma cruzi*. Each slide preparation obtained for the first 47 animals was examined for ten minutes each. The remaining slides were examined for six minutes each. This time proved adequate to cover the slides reasonably well. Routine examinations of the slides were carried out at 400 diameters. Suspicious areas were examined under oil immersion (930 x).

All suggestive bodies were compared with known parasites on slides containing the various life stages of *T. cruzi* provided by the United States Public Health Service, Technical Development Laboratories, Savannah, Georgia and by the State of Oklahoma Public Health Laboratories in Oklahoma City. Dr. D. W. Twohy provided slides of *T. lewisi*, a common parasite of rodents vectored by a flea (*Nosopsyllus*), and slides of *Leishmania donovani*.

CHAPTER IV

DISCUSSION

The state of Oklahoma was extensively trapped during a period from September 1956 to September 1957 for wood rats, cotton rats and peromyscine mice. None of the rodents trapped were found infected with *Trypanosoma cruzi*. Only three specimens of reduviids (*Triatoma sanguisuga*) were obtained of which two were alive and neither were infected with *T. cruzi*. Twenty rodent nests were examined for the presence of reduviids and none were found.

The results of this study do not preclude the possibility of the occurrence of the disease in Oklahoma. It is believed, although not substantiated at this time, that the disease exists either at a low level of incidence with an isolated distribution, or it is absent entirely at this time. Sullivan et al. (1949) showed a more or less continuous incidence in southern Texas on a line even with the base of the Texas Panhandle extending across the state and southward to the Rio Grande River with the heaviest concentration in the southeastern corner of that state. In northern Texas the disease incidence is spotty. If this is the case and the disease occurs in Oklahoma, it would probably be isolated in its distribution much as it is in northern Texas and could be missed in the sampling since the southern counties of Oklahoma were not extensively trapped.

The terrain of northern Texas as well as the climate is not very different from that of southern Oklahoma (See Appendix). Climatological data were obtained and compared for Wichita, Kansas; Fort Smith, Arkansas; Tulsa and Oklahoma City, Oklahoma; Amarillo, Dallas and Wichita Falls, Texas. No essential differences were noted between the cities in Oklahoma when compared with other cities in the neighboring states on an individual basis. These data included the maximum-minimum monthly average temperatures, monthly average relative humidity, prevailing wind direction, and the wind force by month (See Appendix). It is apparent that the vectors could migrate into Oklahoma with the prevailing southerly winds from areas of infection in Texas. Dallas was included in this comparison of weather data because the rodents (Neotoma spp.) have been found infected in that area. Wood (1950a) quoting a letter from a Mr. W. J. Cummings, dated February 1949 states that conditions reported in this letter originating in the Nogales, Arizona area were:

> "Day after day the fire weather forecast read, humidity critically low. Instead of beginning to gather Triatoma specimens in the latter part of April a very few began to appear the last of May. This stopped entirely until we had two days when the humidity rose to a point where one could smell it. During that time I was able to gather a number of specimens for the State Health Department..... The humidity then dropped clear out of sight and with it Triatoma disappeared until our summer rains became normal. The Triatoma responded and for a few days they were crawling all over the camp....".

If the author of that letter meant that the humidity reached a level of say 70% this would run counter to the data presented here. It would seem probably that the humidity did not exceed a level of 50% due to the location of Nogales, Arizona where the annual precipitation average is only 5 inches or less (Encyclopaedia Britannica, World Atlas, 1955). In this case the data would roughly correspond as to incidence of triatomine bugs. The incidence of Triatoma sanguisuga was reportedly high during the years 1955 and 1956. These two years were the last of a drought period in the Southwest. The humidity reached a low level during October 1956 as recorded by the United States Weather Bureau at the Oklahoma City Airport. A distinct rise in the relative humidity will be noted for Tulsa and Oklahoma City in the year 1957 (See Appendix). On the other hand the populations might fluctuate with the ten year drought cycle common to Oklahoma.

The overall trapping success of nearly 10.0% indicates a ready acceptance of the bait to the rodents sought.

The two types of traps worked equally well under all field conditions, however the traps of special design were easier to set and transport because of their size. These traps were occasionally tripped prematurely by large wood rats as they entered the traps, due to their size and hunched backed posture. The falling door would then hit them in the middle of their backs and cause the rodent to back out of the trap and escape.

Drs. Howell and Hopla of the Oklahoma State University and the University of Oklahoma, respectively, have stated that during the years 1955 and 1956 numerous communications were received requesting information on the presence and control of these triatomine bugs in households throughout the state. Dr. Hopla further reported that trypanosomes were found by him in Triatoma sanguisuga in Woods County, Oklahoma during the summer of 1955. Although the trypanosomes were not identified the chance cannot be overlooked that they might have been T. cruzi since this is the common trypanosome in this species of bug.

The Oklahoma Pest Control Association members have not forwarded any specimens of reduviids to the author and his failure to find many specimens leads one to conclude that the population levels of this pest are low. The relative abundance of bugs in former years contrasted with 1957 might indicate a cyclic population based upon climate and other undetermined factors.

A low virulence of the disease within the United States, the difference between feeding and defecation time, and climatic factors producing possible cyclic populations may have restricted the northward spread of the disease.

CHAPTER V

SUMMARY AND CONCLUSIONS

The discovery of a human case of American trypanosomiasis in Texas by Woody and Woody (1955), and the subsequent second case in the same state stimulated workers afresh to find out more about the malady within the borders of the United States. A field investigation was carried out for one year to determine whether the disease was present among certain wild rodents in Oklahoma, principally Neotoma spp., Peromyscus spp., and Sigmodon hispidus. Trapping operations were carried out during the period of September 30, 1956, to September 30, 1957. Trapping sites were located in at least one location in 21 of the 77 counties in Oklahoma. A total of 1525 trap nights with a successful trapping percentage of 9.9% resulted in a catch of 151 animals of which 133 were examined for trypanosomes. No trypanosomes were found indicating a possible low level of infection, if the disease were present at all in this state. Only two live specimens of Triatoma sanguisuga were captured and examined for the etiological agent of this disease. In addition 20 rodent nests were examined for cone nosed bugs and none were found.

The results of this survey do not preclude the possibility of the disease being found in Oklahoma. It is believed that the disease could be present, and if this be the case, it would be at a low level of incidence and isolated in its distribution within the state. It is possible that the hosts examined may not be the reservoirs here in this state since the host range is rather wide (refer to Table II). It has been shown, however, that the disease, as an enzootic, classically is found in the sylvan rodents within the United States. Since terrain factors and climatological data do not show any essential differences between northern Texas and southern Oklahoma and the fact that trypanosomes have been found on one occasion in this state, there is a possibility that the disease may still be discovered as an enzootic among wild animals of this state. The distribution of the vectors seems to be closely associated with climatic factors and possibly other undetermined factors.

CHAPTER VI

SUGGESTIONS FOR FURTHER STUDY

Upon looking at this study in retrospect there should have been more emphasis placed upon such possible reservoirs of the disease as the opossum, *Didelphis virginiana* Kerr, the racoon, *Procyon lotor* (Linn.), the domestic rodents and the armadillo *Dasypus sp.* . Although some work has been done in this state on the role of the bat in trypanosomiasis, this work should be extended to other parasites such as the ticks of the genus *Ornithodoros*, which have members that are parasitic upon bats. Some members of this genus have been artificially infected with *T. cruzi* (Usinger 1944, Matheson 1950). Since streblids (Diptera: Streblidae) are also parasitic upon bats and thus come in close contact with the ticks (*Ornithodoros*) these flies should be surveyed to determine whether the trypanosomes of *T. cruzi* might be harbored in these insects.

It is recommended that before another student attempts a study of this kind that some indices of abundance of vectors be established before the study is begun. Should this line of investigation be pursued in the future a more thorough coverage of the southern counties of Oklahoma should be undertaken initially, and if possible the study should be extended over two or more summers.

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APPENDIX

TABLE VI

CLIMATOLOGICAL DATA

Oklahoma City, Oklahoma

MONTHLY AVERAGE

Prevailing Wind Direction

Wind Force (mph)

LEGEND
_____Maximum Temp.
_x_x_Minimum Temp.
-o-o-o-Relative Humidity

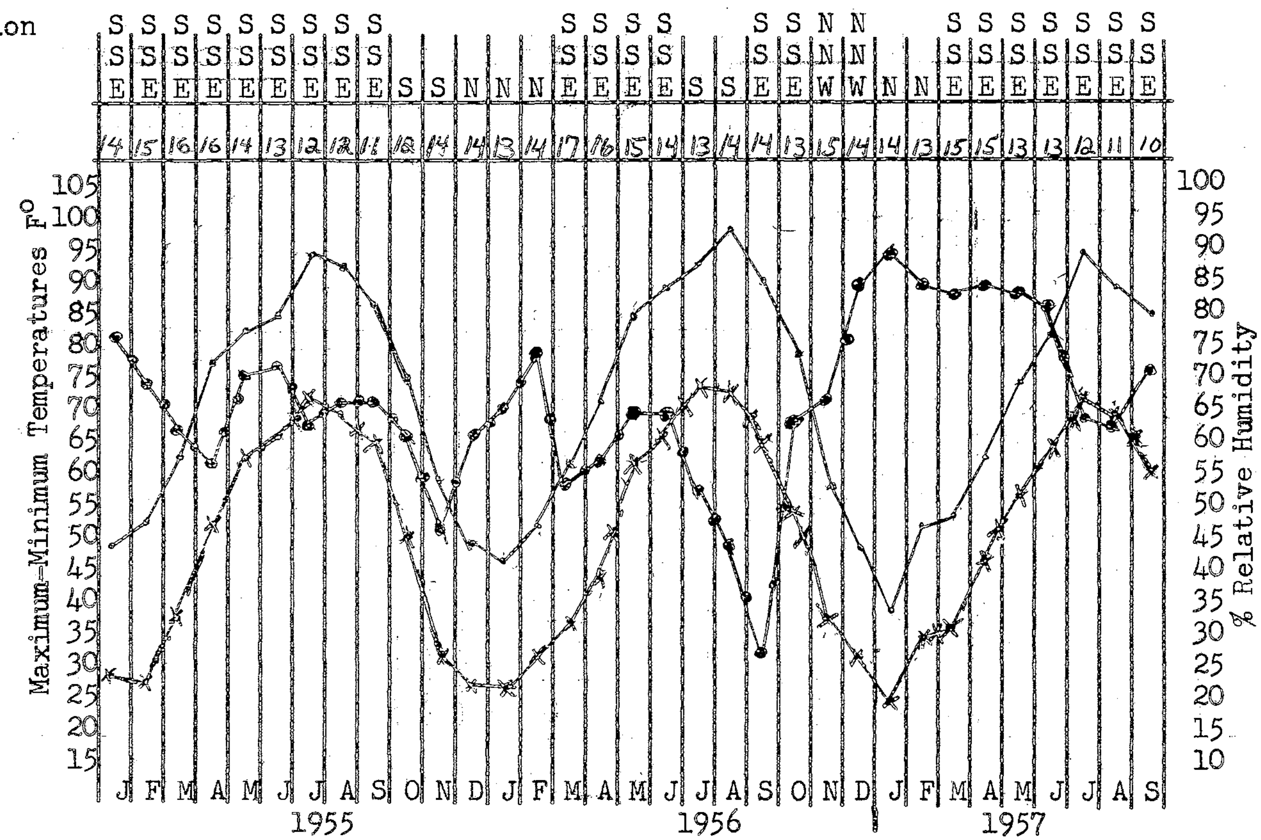


TABLE VII

CLIMATOLOGICAL DATA

Tulsa, Oklahoma

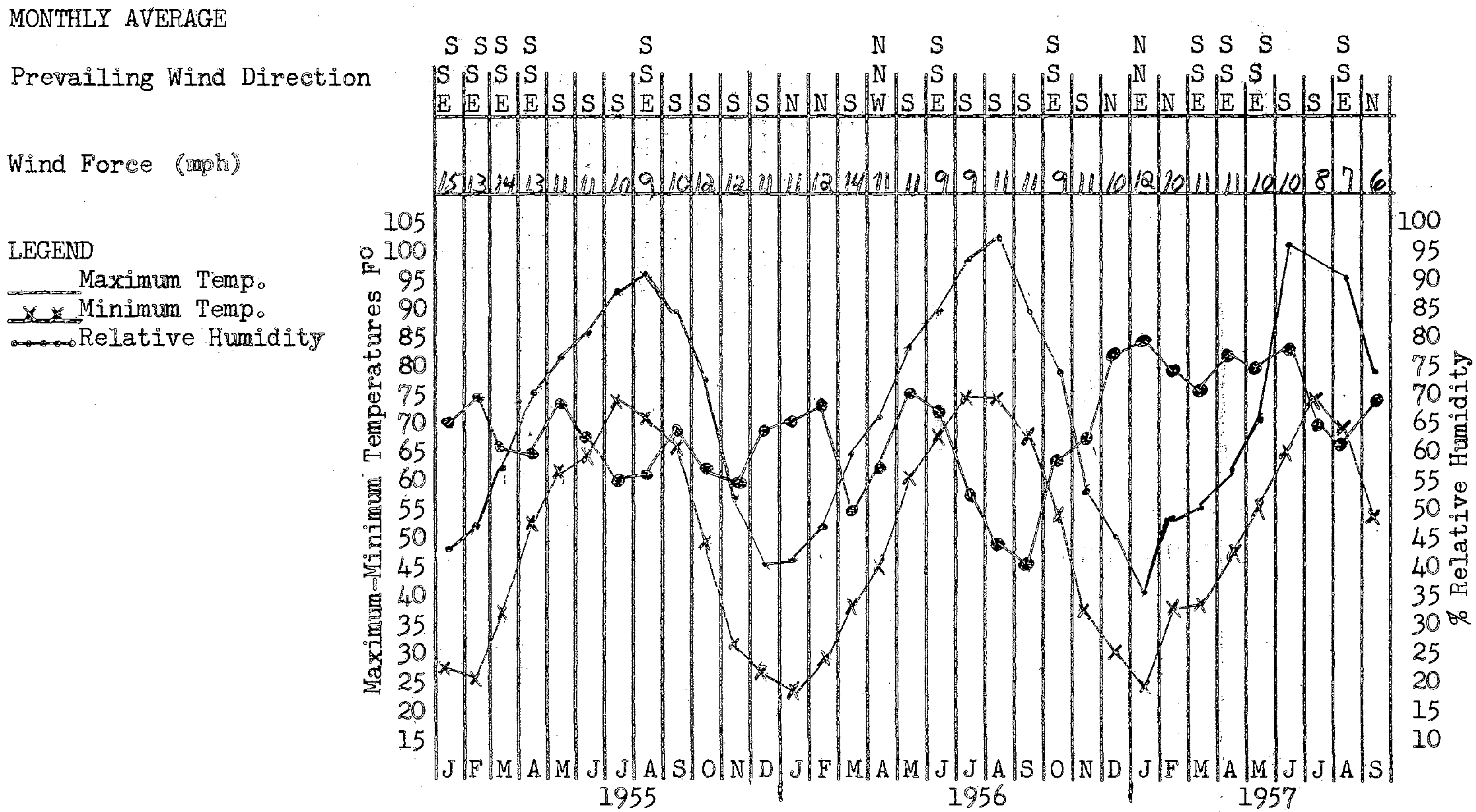


TABLE VIII

CLIMATOLOGICAL DATA

Wichita, Kansas

MONTHLY AVERAGE

Prevailing Wind Direction

Wind Force (mph)

LEGEND
_____Maximum Temp.
_o_o_Minimum Temp.
-o-o-o-Relative Humidity

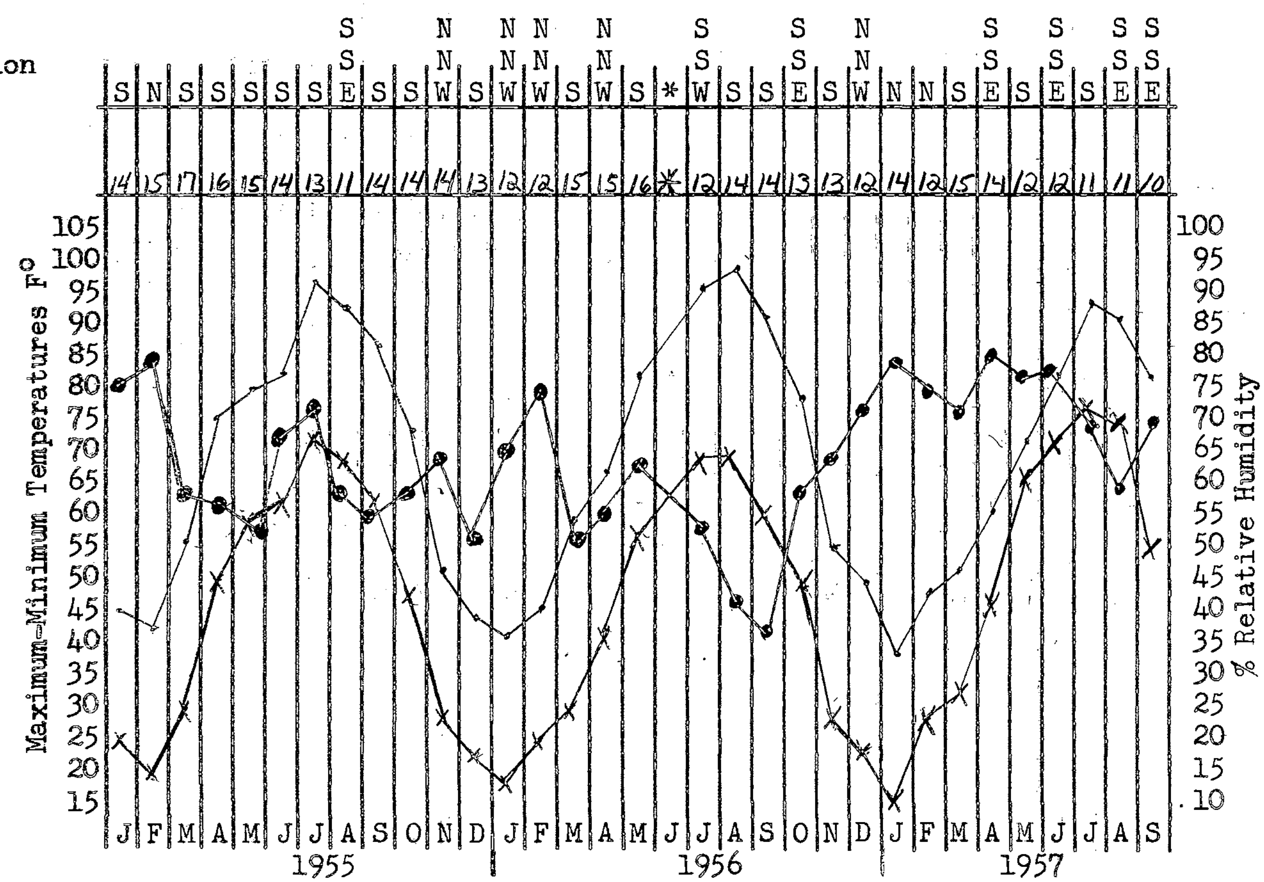


TABLE IX

CLIMATOLOGICAL DATA

Ft. Smith, Arkansas

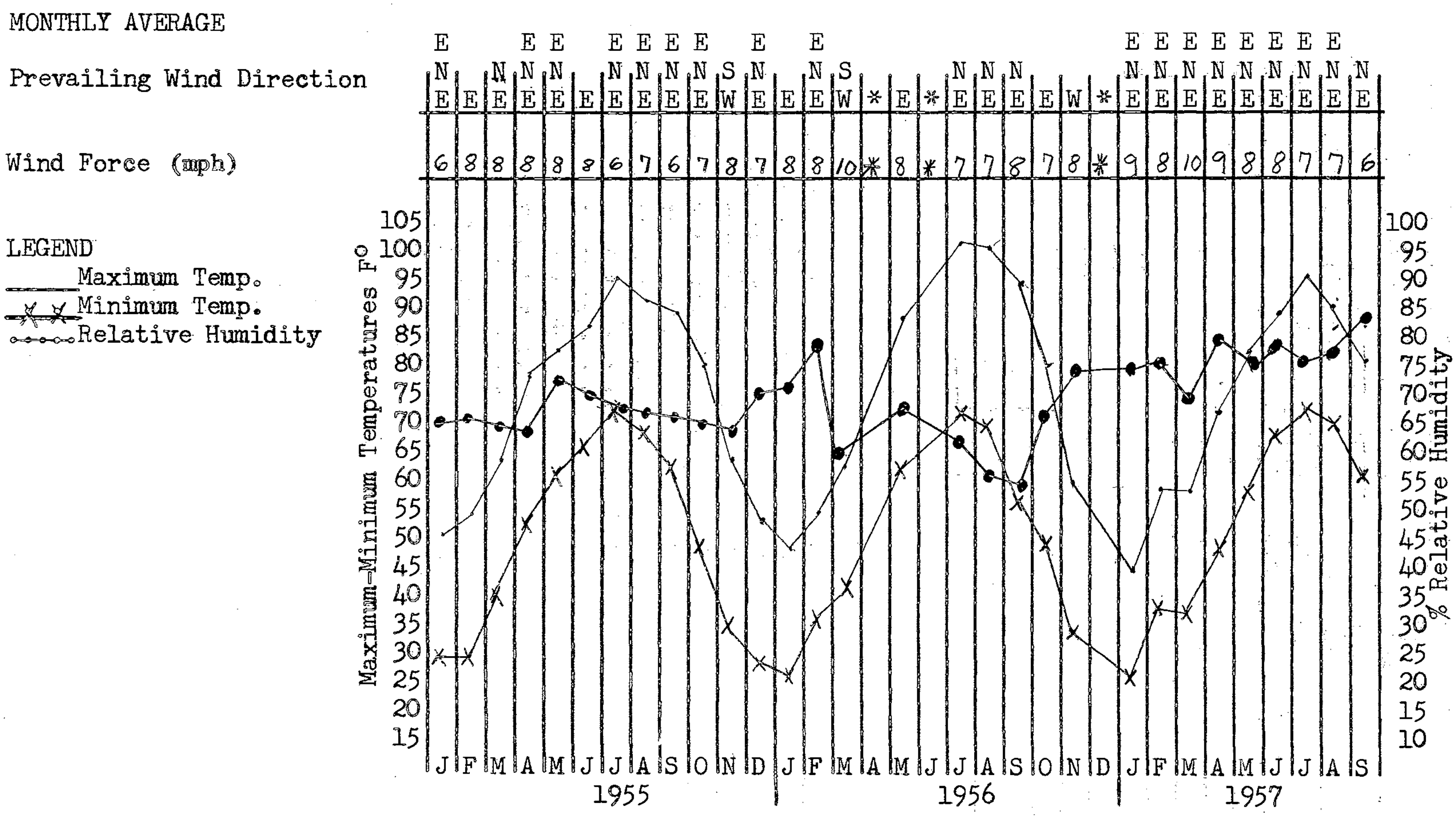


TABLE X

CLIMATOLOGICAL DATA

Amarillo, Texas

MONTHLY AVERAGE

Prevailing Wind Direction

Wind Force (mph)

	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S
Prevailing Wind Direction	SE	SW	SW	SW	S	SSE	S	SSE	S	SW	SW	SW	S	SW	SW	SW	SSW	S	S	S	S	S	SW	SW	SW	SSW	NW	SW	S	S	S	S	S
Wind Force (mph)	14	16	15	16	14	16	13	10	12	12	15	14	12	15	17	18	19	17	15	15	17	16	12	11	14	13	15	14	13	12	11	11	11

LEGEND
____ Maximum Temp.
-x-x- Minimum Temp.
-o-o-o- Relative Humidity

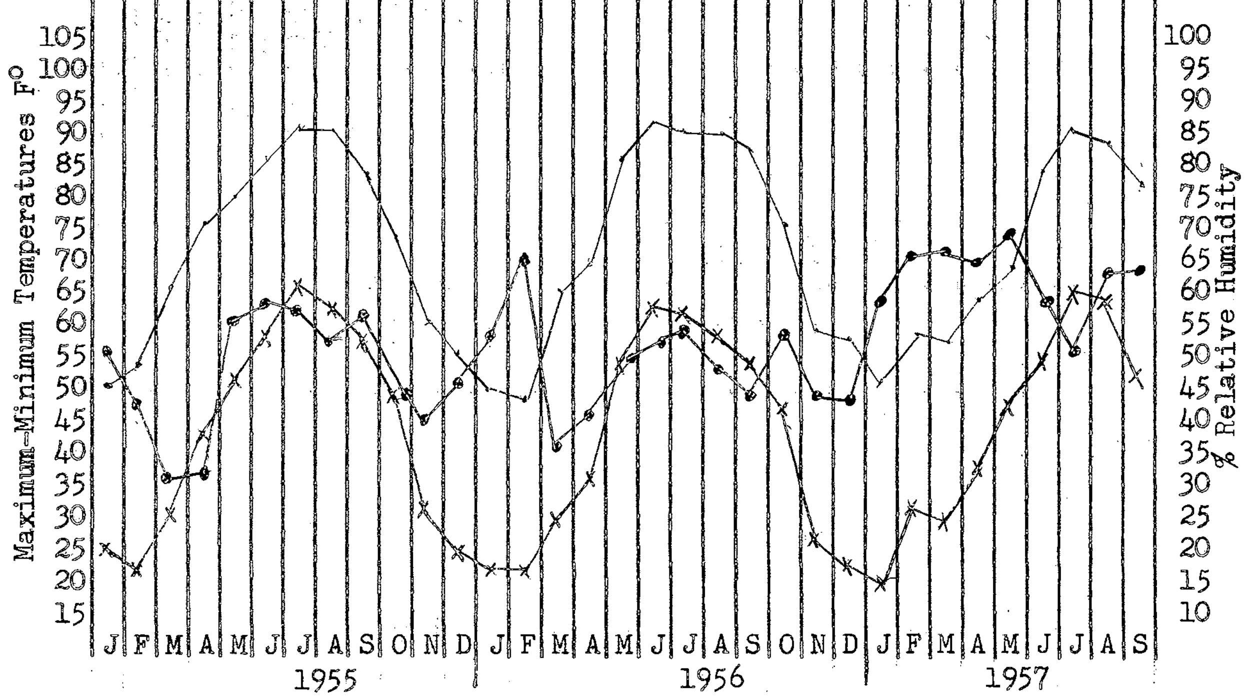


TABLE XI

CLIMATOLOGICAL DATA

Dallas, Texas

MONTHLY AVERAGE

Prevailing Wind Direction

Wind Force (mph)

LEGEND
______Maximum Temp.
-x-x-Minimum Temp.
-o-o-o-Relative Humidity

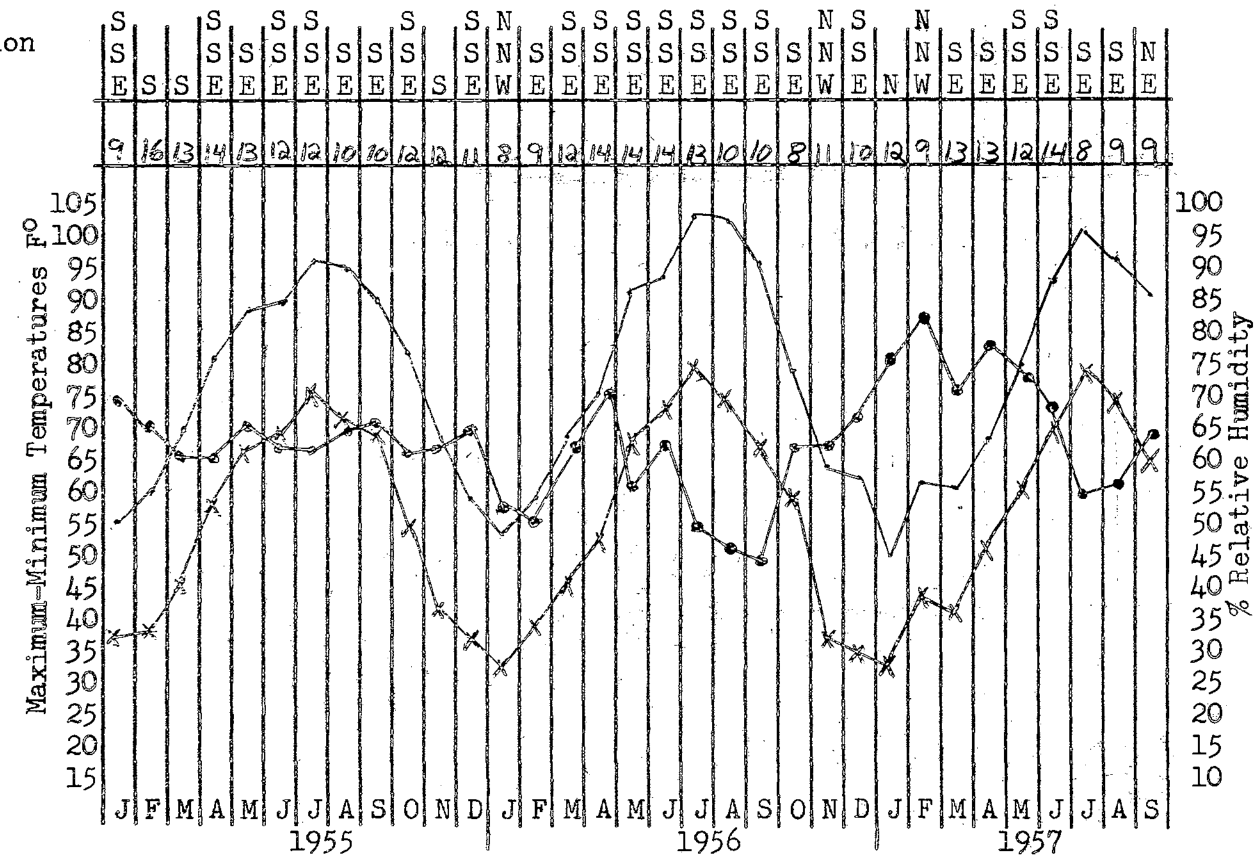


TABLE XII

CLIMATOLOGICAL DATA

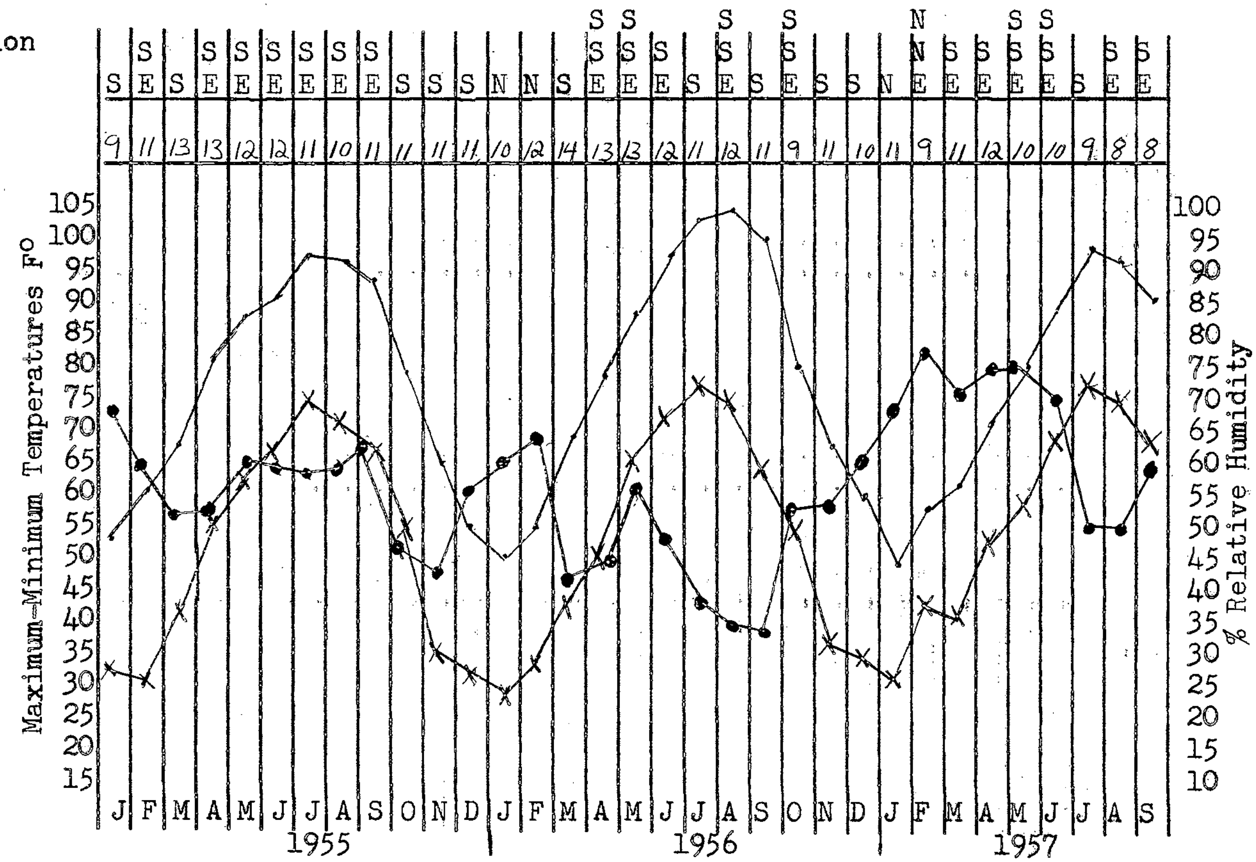
Wichita Falls, Texas

MONTHLY AVERAGE

Prevailing Wind Direction

Wind Force (mph)

LEGEND
_____ Maximum Temp.
-x-x- Minimum Temp.
-o-o-o- Relative Humidity



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