DISTRIBUTION, SEASONAL PARITY AND OVARIAN DEVELOPMENT IN MAJOR PEST SPECIES OF OKLAHOMA TABANIDAE (DIPTERA)

By

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CHAPTER I

INTRODUCTION

The tabanids (Diptera:Tabanidae) are a large family of haematophagous flies, many of which are aggressive biters of man, livestock and wild animals in North America and other parts of the world. Some species of horse flies and deer flies are known to mechanically transmit pathogenic disease agents to domestic animals (Krinsky 1976) and an isolate of a human encephalitis virus from a tabanid species (Wright et al. 1970) has heightened interest in their role as vectors of human disease in North America. Horse flies are important pests of livestock primarily because of the large quantities of blood lost to their feeding. Studies have been made on the volume of blood imbibed by several species (Tashiro and Schwardt 1949; Gooding 1972; Hollander and Wright 1980b), and while there are no reliable estimates of total losses caused by horse fly attacks, Hollander and Wright (1980b) reported that a majority of tabanid species examined at least doubled their body weight after a blood meal.

Estimates on the economic losses to livestock by tabanid feeding worldwide have been reported to be as high as $40 million annually (Steelman 1976). Bruce and Decker (1951), Granett and Hansens (1956) and Roberts and Pund (1974) reported the detrimental effects of horse flies on weight gains and milk production. Because of their general annoyance to man, control of the Tabanidae is an active concern of researchers and vector control agencies in several states (Catts 1971).

Knowledge on reproductive biology could be important for determining the effectiveness of control procedures for the Tabanidae. Because of the ability of horse flies to feed rapidly, the effects of control measures will not always be immediately recognized since damage to the host will have already been inflicted. Benefits of control will come through the death of the fly, before oviposition can take place.

One factor that could influence the impact of a tabanid species would be the presence of autogeny in that species. Autogeny is a term used to denote egg production in female insects which have not fed upon blood. Anautogeny, on the other hand, applies to the more common, obligate blood feeding forms of haematophagous Diptera. After the completion of a gonotrophic, or egg laying cycle, a female is described as being parous. The term nulliparous, therefore, is applied to females which have not produced their first batch of mature eggs.

Age determination techniques in haematophagous Diptera were first developed by Polovodova in Russia and were described by Detinova (1962). The technique involves the excision and examination of the ovaries for follicular artifacts which consist of dilatations, or thickenings, in the proximal portion of the ovarioles (Bertram 1962). Most of the dilatations are small relics, called "yellow bodies", that are made up of cellular debris from the follicular epithelium (Thomas 1972, 1973). This technique has been used to determine if autogeny exists in tabanid species by numerous workers (Anderson 1971; Bosler and Hansens 1974; Duke 1959; Lewis 1960; Lutta 1964; Magnarelli 1976; Magnarelli and Pechuman 1975; Morris and DeFoliart 1971; Raybould 1967; Rockel 1969). Some of the different species of horse flies and deer flies which have already been reported to be autogenous include Tabanus iyoensis Shiraki

(Saito 1967; Watanabe and Kamimura 1971), Chrysops atlanticus Pechuman (Anderson 1971), T. nigrovittatus Macquart (Magnarelli and Anderson 1977), Atylotus incisuralis (Macquart), Hybomitra osburni (Hine), H. frontalis (Walker), H. itasca (Philip), H. liorhina (Philip) and H. rupestris (McDunnough)(Thomas 1972).

Several tabanid species found in Oklahoma have been demonstrated to be anautogenous for the first ovarian cycle in other geographical areas. These include T. lineola Fabricius (Rockel 1969), H. lasiophthalma (Macquart)(Thomas 1972; Morris and DeFoliart 1971), T. sulcifrons Macquart, T. sparus milleri Whitney and C. callidus Osten Sacken (Magnarelli 1976). In this investigation, T. abactor Philip, T. mularis Stone, T. subsimilis Bellardi and T. sulcifrons were analyzed for parity since they had previously been reported as major pests of cattle (Hollander 1979; Schomberg 1952; Schomberg and Howell 1955). A series of laboratory and field trials were also undertaken using engorged female Tabanus species to determine the length of time between feeding, egg production, oviposition and subsequent host seeking activities. It is hoped that data from this latter study may shed some light on the number of gonotrophic cycles which Tabanidae undergo and the corresponding epidemiologic significance of their feeding habits.

Studies on distribution, reproduction and economic importance of Tabanidae rely on some type of trap to monitor populations, and there are an ample number of trap designs, modifications and methods from which to choose depending upon one's sampling environment and needs. Roberts (1976) reported that a "Stoneville" Malaise trap constructed of natural Saran® screen and baited with CO_2 collected more tabanids than any of several other trap types which were tested. Studies have also

shown that providing a source of CO_2, either with bottled gas or by sublimating dry ice, will significantly increase the number of tabanids collected (Anderson et al. 1974; Blume et al. 1972; DeFoliart and Morris 1967; Everett and Lancaster 1968; Olkowski and Anderson 1967; Roberts 1970, 1971, 1972, 1975, 1976, 1977; Wilson et al. 1966). Hollander and Wright (1980a) made correlations of the number of horse flies collected in CO_2 baited Malaise traps with the numbers observed attacking a cow, and in seven species probabilities ranging from 91% to >99% showed that traps were accurately measuring daily activity of the flies.

In the present study, trap collections of Tabanidae were made in a cattle producing area of southeastern Oklahoma so that species, relative abundance and temporal activity patterns could be compared to similar data reported for north central Oklahoma (Hollander 1979). To further investigate the significance of pest species encountered, dissections were made on females to determine levels of parity, especially during that period when flies were first collected for the season.

CHAPTER II

SEASONAL OCCURRENCE AND DAILY ACTIVITY CYCLES OF TABANIDAE IN SOUTHEAST OKLAHOMA

Introduction

Howell and Schomberg (1955) listed 70 species of Tabanidae for Oklahoma, including several which were new state records at that time, but did not include collection localities. In a taxonomic review of the Tabanidae of Louisiana, Tidwell (1973) details the life history, ecology and collection data for 100 species and subspecies of that state including 18 species which occur in Oklahoma. Hollander (1979) reported collecting 21 species in north central Oklahoma. Eight species, H. lasiophthalma, T. abactor, T. atratus Fabricius, T. equalis Hine, T. mularis, T. sulcifrons and T. trimaculatus Palisot de Beauvois, were very abundant and important pests of cattle.

The purpose of this study was to determine the seasonal abundance, daily activity patterns and distribution of tabanid species at the Kerr Foundation Ranch in southeastern Oklahoma using standard flight traps baited with CO_2, and by making visual observations of horse flies attacking cattle. This information was compared with similar data concerning the Tabanidae in north central Oklahoma (Hollander 1979).

Materials and Methods

Trapping Location

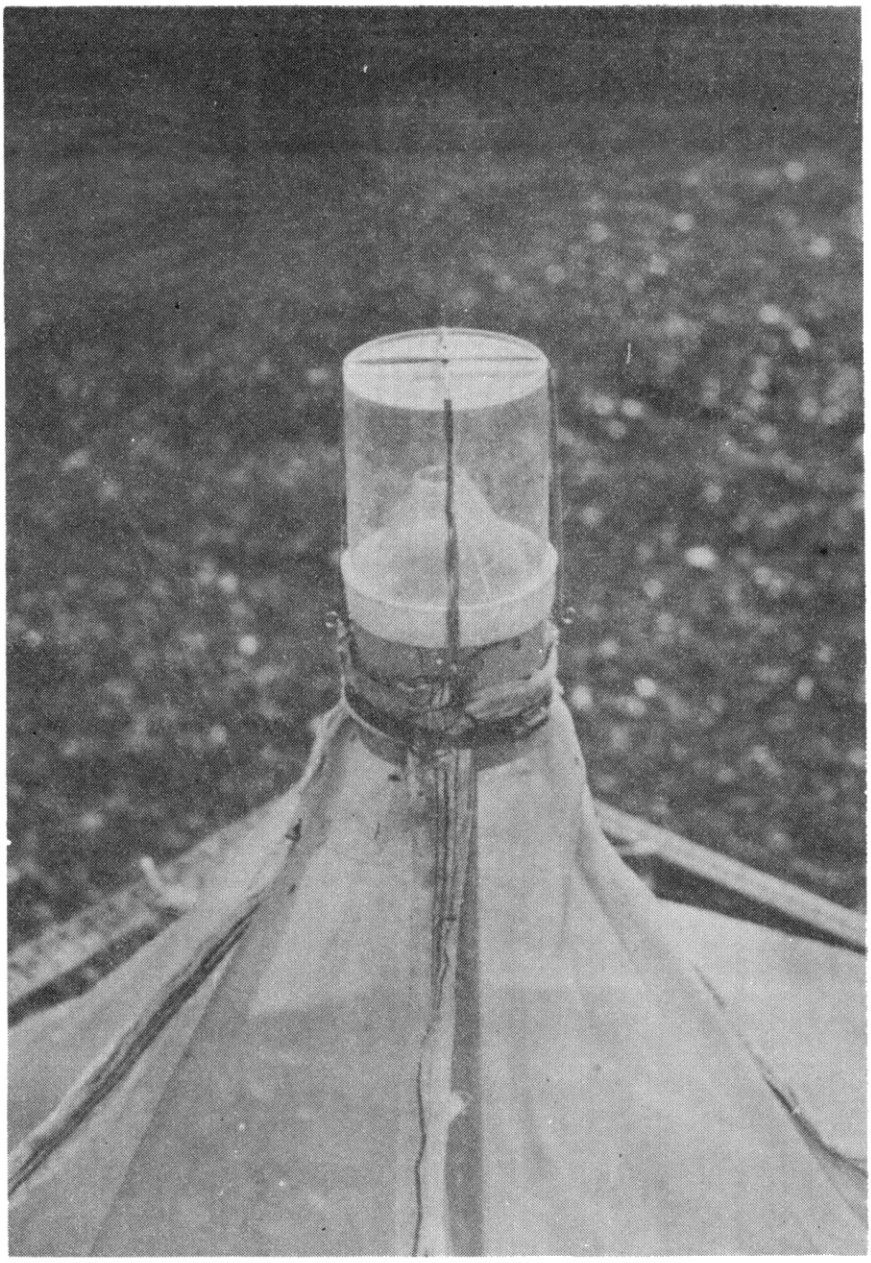
Tabanid surveys were conducted from March 30 through October 11, 1979, on a four hectare site of the Kerr Foundation Ranch, south southwest of Poteau in LeFlore County, Oklahoma. Topography near the trap sites consisted mainly of open pasture lands interspersed with several types of hardwood trees. Two farm ponds were situated in the upper two hectare tract and covered approximately 0.1 and 0.3 hectares, respectively. The lower two hectares of the survey site had a moderately fast flowing stream along it's southern border.

Two permanent trap sites were established in the lower two hectare tract and were separated by about 914 meters. One trap was in a low region, ten meters from a stream, and faced open pasture areas on three sides. It was also subjected to direct sunlight for four to six hours per day. The second trap was situated on a slight rise in a moderately wooded area and received little direct sunlight. All observations of tabanids coming to cattle were made in the upper two hectare tract near a barn which was located 1829 meters equidistant from each trap.

Trapping Procedures

Traps utilized in this study were similar to a modified "Stoneville" Malaise trap described by Roberts (1976), but were reinforced by the addition of a steel frame (Hollander 1979). Collection tops were made out of one liter, clear Nalgene® polypropylene jars with screw top lids which had been fitted with inverted plastic funnels (Fig. 1). The jars were held in place on the center trap support using rubber bands. A

Figure 1. One liter polypropylene jar with inverted funnel used for collecting tabanids with a modified Malaise trap.



2.54 cm square of dichlorvos resin was placed in each top to rapidly immobilize and kill collected flies.

Malaise traps baited with CO_2 from bottled gas or sublimating dry ice were used to attract tabanids to traps. Gas was continuously released from a 9.1 kg cylinder at the rate of 250 ml/min using a Matheson, Model 8 gas regulator. The rate of flow was calibrated every three hours with a Gilmont Model F-2060-A, shielded flowmeter. The traps were run for one 24 hour period beginning at 6 PM. Trap catches were retrieved every three hours, i.e., 9 AM, 12 noon, 3 PM, 6 PM and 9 PM to define peak activity periods. Continuous trapping was also conducted from 9 PM to 6 AM to complete a full 24 hour cycle and stored until individual flies could be separated to species and counted.

During alternate weeks in this study, 24 hour trap collections were made, but no observations were conducted on cattle. On these occasions, flight traps were baited using 4.5 to 5.4 kg of dry ice held in styrofoam buckets which had holes punched in the walls to allow sublimating CO_2 gas to escape. A trap top with dichlorvos resin was mounted on the trap and removed at the end of the 24 hour period.

Observations of Tabanids on Cattle

Observations were made of tabanid host seeking activity on Black Angus cattle from 6 AM until 9 PM every other week of the survey period. A small herd of approximately 20-25 cows were fenced off in the upper two hectare tract of the barn site while CO_2 trapping was conducted. For ten minutes each hour, the number of tabanids by species was recorded along with the landing site of the animal. A pair of 7 X 50 binoculars was used in the identification of flies, and when possible,

the same animal was viewed for the duration of the ten minute survey. Viewing distances varied from five to ten meters.

Results and Discussion

Species Present

Thirty two species of Tabanidae were collected in traps on the Kerr Foundation Ranch, Poteau, Oklahoma (Table I) between April 14 and October 5. Three species, T. subsimilis, T. mularis and T. sulcifrons, accounted for >78% of the total collected (Table I). These three species were also observed attacking cows frequently and were obviously major pests of cattle in the area. Five other species accounted for 12% of the total number collected, and although they attacked cattle they were not abundant enough to be considered major pests. These species were T. sparus milleri, T. sublongus Stone, T. abdominalis Fabricius, T. abactor and H. lasiophthalma. Additionally, a few T. trimaculatus, T. atratus, T. quinquevittatus Wiedemann and T. equalis were observed on cattle. Chrysops species were observed coming to cattle periodically, but a species determination was not made. Nine species of Chrysops were trapped, of which C. sequax Williston was the most abundant, but accounted for only 1.17% of the total tabanids collected.

Few specimens of Chlorotabanus crepuscularis (Bequaert), H. nigricans (Wiedemann), Leucotabanus annulatus (Say), T. americanus Forster, T. calens Linnaeus, T. colon Thunberg, T. cymatophorus Osten Sacken, T. lineola, T. melanocerus Wiedemann, T. molestus Say and T. venustus Osten Sacken were captured and were never observed on cattle.

TABLE I

TABANID SPECIES COLLECTED IN TWO MALAISE TRAPS ON THE KERR FOUNDATION RANCH, POTEAU, OKLAHOMA DURING 1979

Species	No. trapped	% of total
Chlorotabanus crepuscularis (Bequaert)	9	< 1
Chrysops callidus Osten Sacken	52	< 1
C. cincticornis Walker	1	< 1
C. flavidus Fairchild	29	< 1
C. moechus Osten Sacken	8	< 1
C. pikei Whitney	27	< 1
C. separatus Hine	25	< 1
C. sequax Williston	109	1.17
C. upsilon Philip	1	< 1
C. wiedemanni Kröber	1	< 1
Hybomitra lasiophthalma (Macquart)	138	1.48
H. nigricans (Wiedemann)	2	< 1
Leucotabanus annulatus (Say)	2	< 1
Tabanus abactor Philip	214	2.29
T. abdominalis Fabricius	254	2.72
T. americanus Forster	27	< 1
T. atratus Fabricius	17	< 1
T. calens Linnaeus	56	< 1
T. colon Thunberg	66	< 1
T. cymatophorus Osten Sacken	121	1.30
T. equalis Hine	26	< 1
T. lineola Fabricius	3	< 1
T. melanocerus Wiedemann	32	< 1
T. molestus Say	3	< 1
T. mularis Stone	2326	24.95
T. quinquevittatus Wiedemann	128	1.37
T. sparus milleri Whitney	293	3.14
T. sublongus Stone	268	3.0
T. subsimilis Bellardi	3157	33.87
T. sulcifrons Macquart	1877	20.02
T. trimaculatus Palisot de Beauvois	50	< 1
T. venustus Osten Sacken	1	< 1

Trap #1 captured 60% of the total flies, perhaps because it was located in an open area which may have been more accessible to flying tabanids.

Seasonal Occurrence and Abundance

The dates for the first and last record of tabanid activity by species are shown in Table II. Although trapping was initiated on March 30, the first specimens were not captured, nor observed attacking cattle, until April 13. One or more species was active from this time until October 5 in Poteau, Oklahoma. Less than 20 specimens of C. cincticornis Walker, C. upsilon Philip, C. moechus Osten Sacken, C. wiedemanni Kröber, Ch. crepuscularis, H. nigricans, L. annulatus, T. atratus, T. lineola, T. molestus and T. venustus were captured and, therefore, an accurate interpretation of their seasonal cycles could not be determined for this location in southeastern Oklahoma.

Nine species of Chrysops were collected at the Poteau site. One of these, C. upsilon, was confirmed as a new state record (Pechuman personal communication 1980). The most abundant species were C. sequax and C. callidus, which totaled 109 and 52 specimens, respectively. Chrysops species were collected from April 13, when tabanids were first observed, until October 5, the date of the last trapping record. The seasonal activity for all Chrysops species began earlier and extended approximately five to six weeks longer than that described for north central Oklahoma (Hollander 1979) and included six species, C. cincticornis, C. moechus, C. pikei, C. separatus, C. upsilon and C. wiedemanni, which were not present in the north central Oklahoma collections.

H. lasiophthalma was present for only a short period from April 13

TABLE II

DATES OF FIRST AND LAST RECORD OF ACTIVITY OF TABANIDS COLLECTED ON THE KERR FOUNDATION RANCH, POTEAU, OKLAHOMA DURING 1979

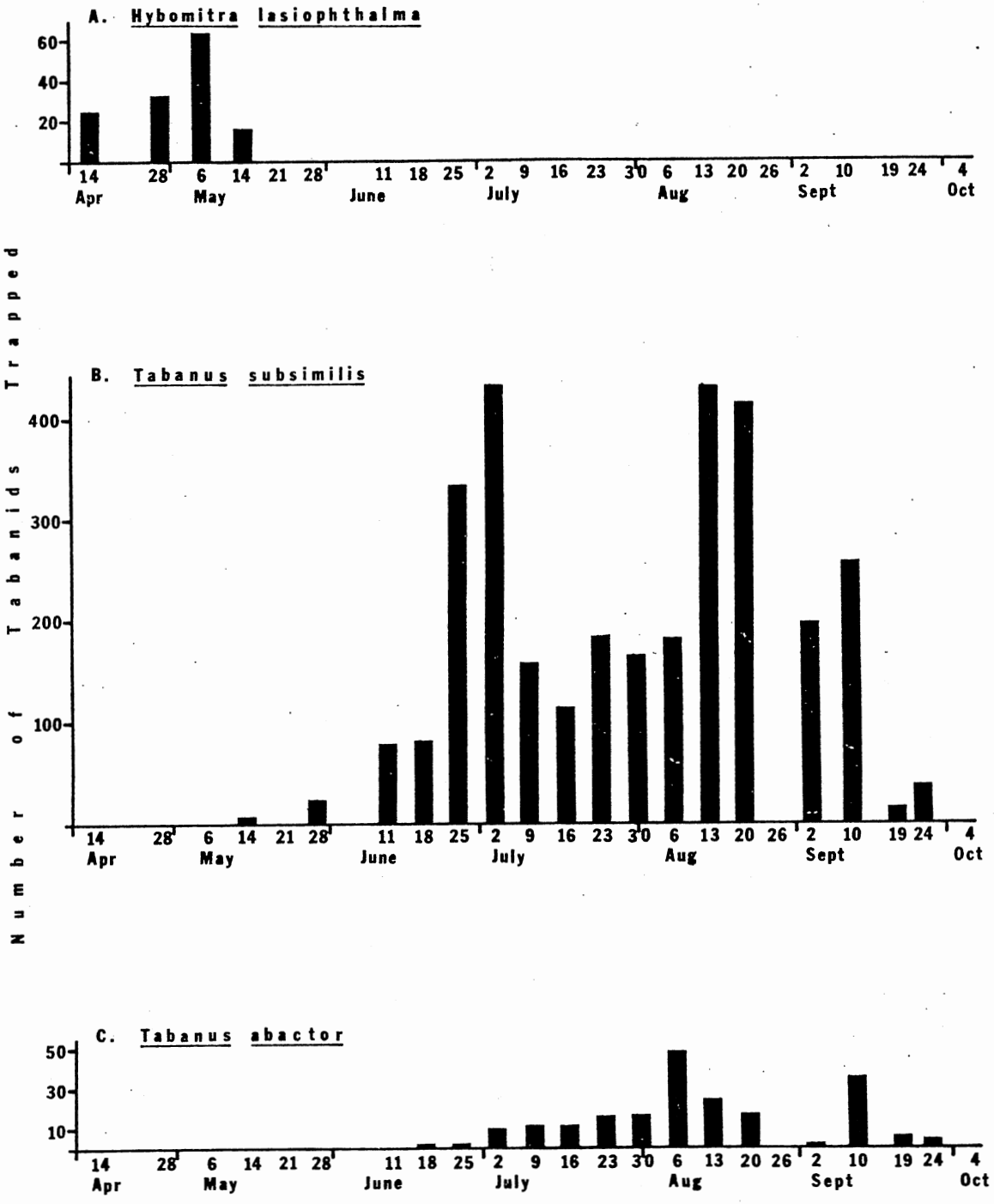
Species	Date	
	First Observed	Last Observed
Chlorotabanus crepuscularis	June 18	September 11
Chrysops callidus	May 5	August 14
C. flavidus	June 11	October 5
C. pikei	May 5	July 17
C. separatus	April 13	May 6
C. sequax	June 18	September 24
Hybomitra lasiophthalma	April 13	May 15
Tabanus abactor	June 18	September 24
T. abdominalis	July 9	August 27
T. americanus	July 2	September 3
T. atratus	June 11	September 24
T. calens	July 23	September 24
T. colon	July 2	September 3
T. cymatophorus	June 25	September 3
T. melanocerus	June 11	August 27
T. mularis	May 14	October 5
T. quinquevittatus	June 11	September 11
T. sparus milleri	May 14	August 7
T. sublongus	June 11	August 7
T. subsimilis	May 14	September 24
T. sulcifrons	June 25	October 5
T. trimaculatus	May 28	August 7

to May 15 (Fig. 2), and most of the 138 females were collected on May 6. Hollander (1979) indicated a similar activity period for H. lasiophthalma in north central Oklahoma, and described the species as a serious pest for the four week period extending from late April to early May. This species did not appear to be a severe pest at the Kerr Foundation Ranch in southeast Oklahoma. Tidwell (1973) reports that H. lasiophthalma was seen in Louisiana from March to May, whereas it's peak activity period was one or two months later in New York (Tashiro and Schwardt 1949, 1953) and Ontario (Golini and Wright 1978).

T. subsimilis was the most abundant species collected, accounting for 33.9% of the total captured. It was first observed in traps and on cattle on May 14 and remained active until September 24 (Fig. 2). T. subsimilis had two major peaks of activity, one on July 2 and another August 13. It was possible that these peaks represent two generations of this species, as T. subsimilis was reared from egg to adult in our laboratory in less than four months. Similar activity peaks were shown by Hollander (1979), although this species was only the fourth most abundant species in north central Oklahoma.

T. abactor was reported to be the most abundant pest species in north central Oklahoma (Hollander 1979). In the Poteau area, T. abactor was only the seventh most abundant species and accounted for less than 3% of the total flies captured (Fig. 2). T. abactor first appeared on June 18 and was active until September 24. This species had two population peaks, i.e., August 13 and September 10, which were later than the June and August peaks reported for this species in the north central region of the state. Trapping records from this study which were confirmed by Pechuman (personal communication 1980) indicate that this was the eastern most range of T. abactor.

Figure 2. Total tabanids collected in two Malaise traps on the Kerr Foundation Ranch, Poteau, Oklahoma in 1979. A. *Hybomitra lasiophthalma*; B. *Tabanus subsimilis*; C. *Tabanus abactor*.


A. Hybomitra lasiophthalma
60
40
20
14 28 6 14 21 28 11 18 25 2 9 16 23 30 6 13 20 26 2 10 19 24 4
Apr May June July Aug Sept Oct
Number of Tabanids Trapped
B. Tabanus subsimilis
400
300
200
100
14 28 6 14 21 28 11 18 25 2 9 16 23 30 6 13 20 26 2 10 19 24 4
Apr May June July Aug Sept Oct
C. Tabanus abactor
50
30
10
14 28 6 14 21 28 11 18 25 2 9 16 23 30 6 13 20 26 2 10 19 24 4
Apr May June July Aug Sept Oct

As was the case in north central Oklahoma, T. mularis was the second most abundant species, accounting for 25% of the total captured. T. mularis was trapped from May 14 until October 5 and had one major peak of activity in late June (Fig. 3). It was a serious pest of cattle throughout the survey period which was also the case in north central Oklahoma.

T. sulcifrons, the third most abundant species present at the Kerr Foundation Ranch in southeast Oklahoma, did not appear until June 24 which was similar to it's activity pattern for north central Oklahoma (Fig. 3). This species was most abundant on July 30, but remained active until October 5, similar to reports by Burnett and Hayes (1977) in Alabama. Twenty percent of the tabanids collected in Poteau were T. sulcifrons and they caused much discomfort to cattle which frequently moved to water or dense brush to avoid their blood seeking activity.

Three species, T. sparus milleri, T. sublongus and T. abdominalis, were not present in north central Oklahoma but were fourth, fifth and sixth, respectively, in abundance from collections made in Poteau (Fig. 4). T. sparus milleri emerged on May 14 and was most active for a 30 day period beginning on June 10 during which time it was frequently observed attacking cattle. T. sublongus was trapped during June and July. This species was not observed attacking cattle, but since it was not very abundant overall and closely resembled T. subsimilis, observation methods limited distinguishing it in the field. T. abdominalis emerged on July 9 and was most abundant in the latter part of the month. It was not observed on cattle.

Diurnal Activity Patterns

On the basis of trap collections and observations of flies

Figure 3. Total tabanids collected in two Malaise traps on the Kerr Foundation Ranch, Poteau, Oklahoma in 1979. A. *Tabanus mularis*; B. *Tabanus sulcifrons*.

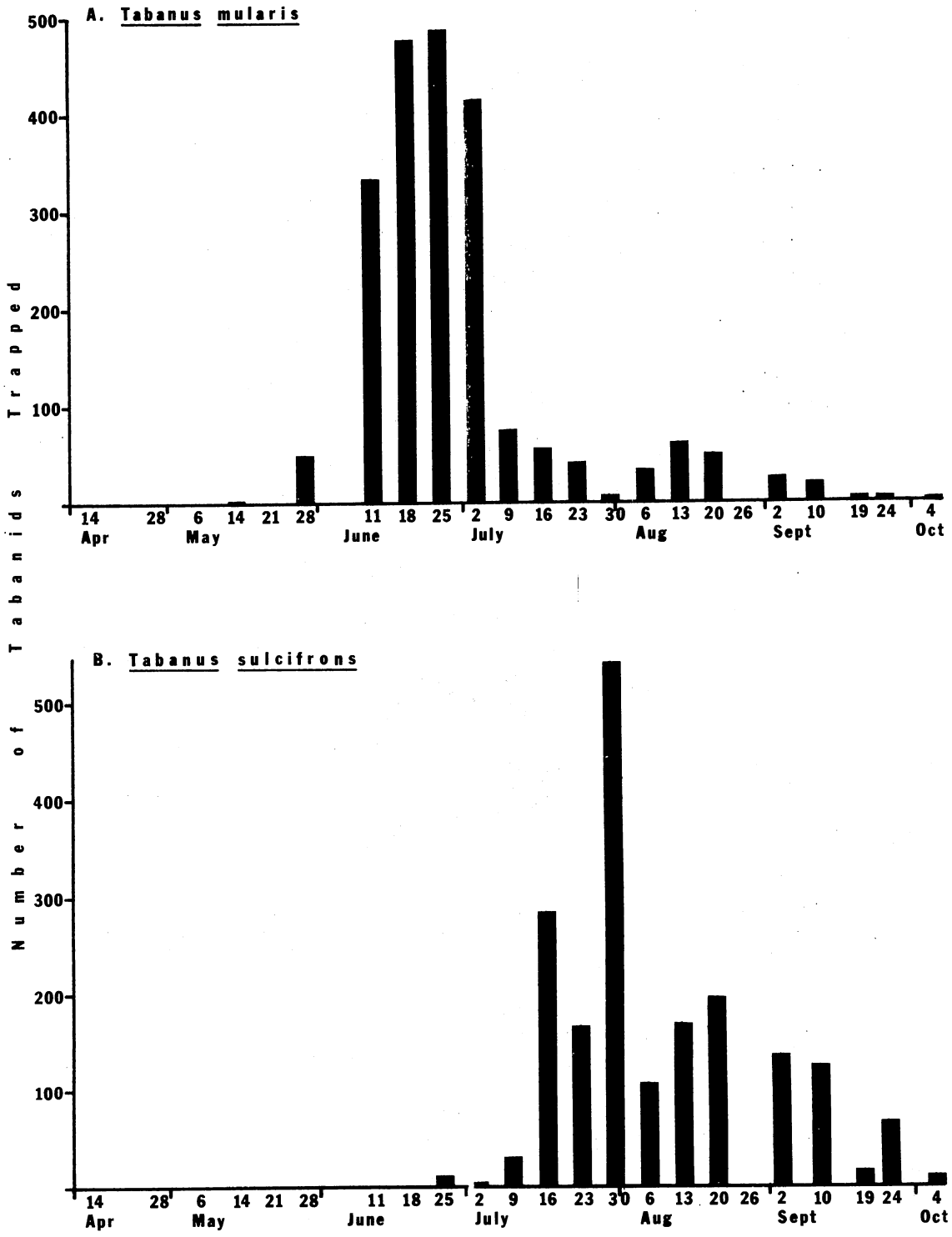
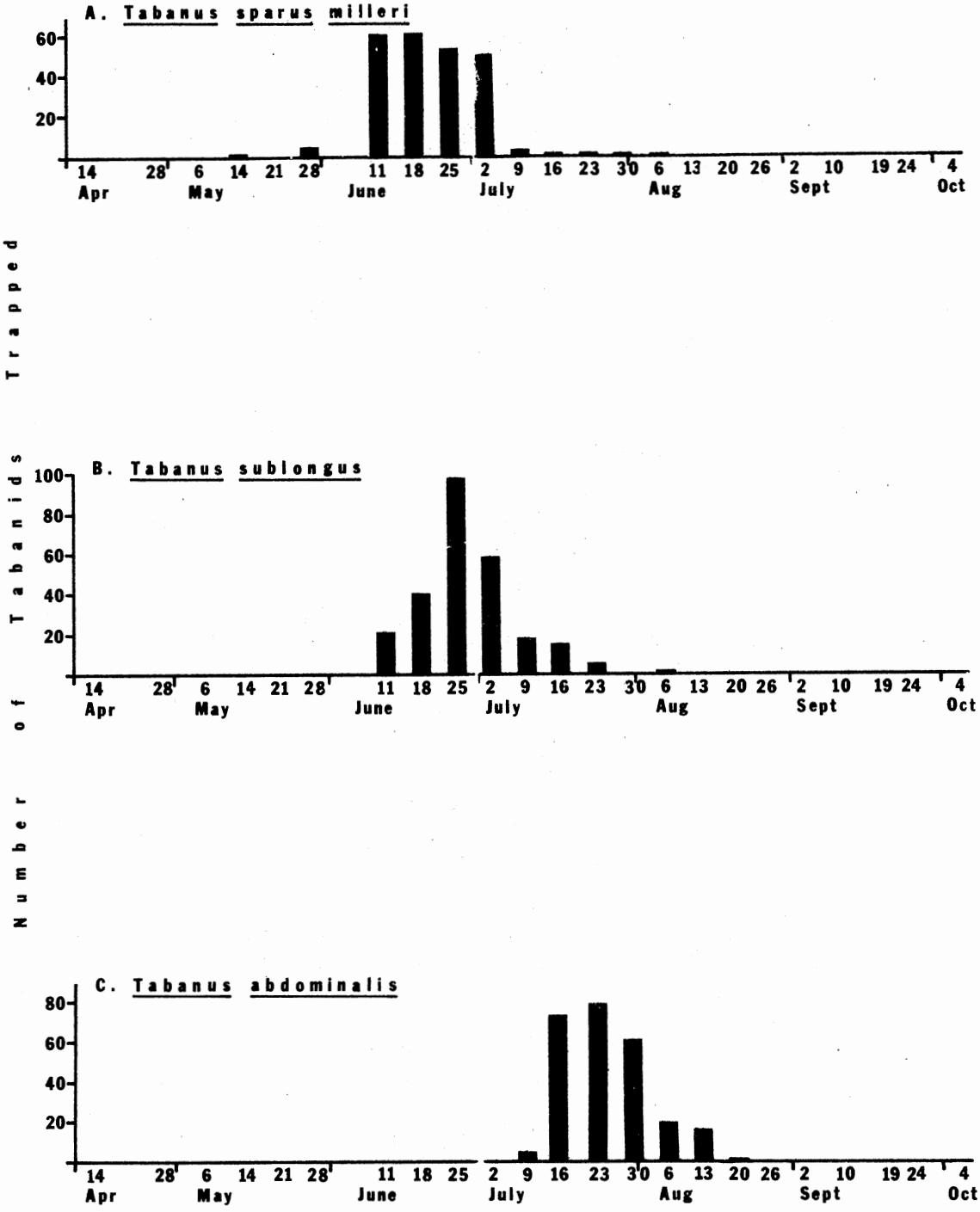

A. Tabanus mularis
B. Tabanus sulcifrons
Number of Tabanids Trapped
500
400
300
200
100
14 28 6 14 21 28 11 18 25 2 9 16 23 30 6 13 20 26 2 10 19 24 4
Apr May June July Aug Sept Oct

Figure 4. Total tabanids collected in two Malaise traps on the Kerr Foundation Ranch, Poteau, Oklahoma in 1979. A. Tabanus sparus milleri; B. Tabanus sublongus; C. Tabanus abdominalis.


A. Tabanus sparus milleri
B. Tabanus sublongus
C. Tabanus abdominalis
Number of Tabanids Trapped
14 28 6 14 21 28 11 18 25 2 9 16 23 30 6 13 20 26 2 10 19 24 4
Apr May June July Aug Sept Oct

attacking cattle, diurnal activity patterns of the eight most abundant species at the Poteau research site were determined. In a species by species comparison, diurnal patterns recorded for these species were similar to that found in north central Oklahoma by Hollander (1979).

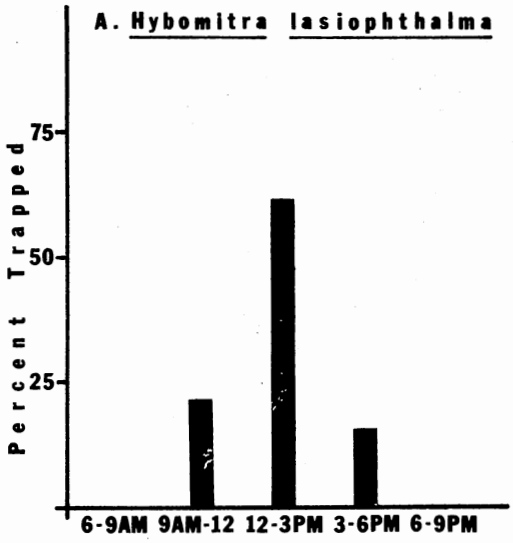
H. lasiophthalma, the first species to appear, was most abundant from noon until 3 PM (Fig. 5). Additionally, there was a small amount of activity, i.e., 16-22% of total trap collections, from 9 AM to noon and 3 PM to 6 PM. H. lasiophthalma was never trapped nor observed coming to cattle before 9 AM or after 6 PM. The number of flies caught at the two traps was approximately the same on all survey dates.

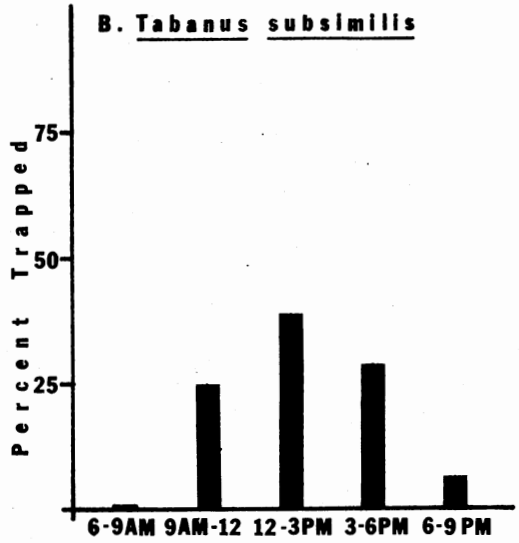
T. subsimilis, the most abundant species collected, was found in traps in a bell shaped curve beginning at 9 AM, with peak activity from noon until 3 PM, and decreased rapidly after 6 PM (Fig. 5). This species was observed attacking cattle in greater numbers, and for longer periods than any other species.

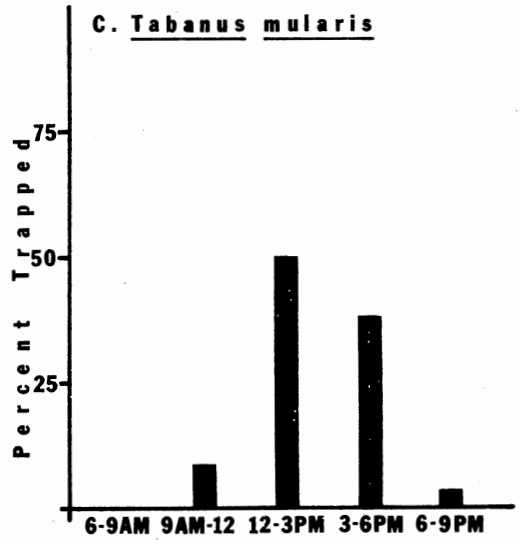
Few T. mularis were trapped before 9 AM or after 6 PM (Fig. 5). The peak period of activity for this species was from noon until 3 PM after which the numbers gradually decreased. The species was captured equally well in both traps and were observed attacking cattle at a similar rate.

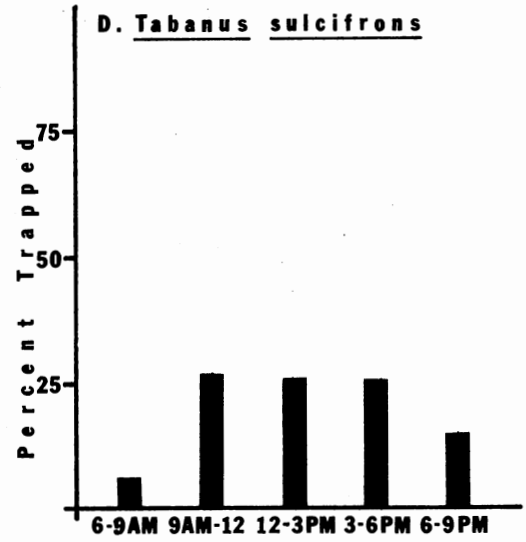
T. sulcifrons was collected from sunrise to sunset with approximately 20% of the specimens captured during the 6 AM to 9 AM and 6 PM to 9 PM period, and the remaining collected evenly throughout the 9 AM to 6 PM period (Fig. 5). More T. sulcifrons were collected in trap #1, perhaps because it was located in a major flight path for this species. The species was most abundant during the latter part of July, but remained active for five to six weeks longer than T. abdominalis, a closely related species.

Figure 5. Percentage of total tabanids collected by three hour trapping periods on the Kerr Foundation Ranch, Poteau, Oklahoma in 1979. A. *Hybomitra lasiophthalma*; B. *Tabanus subsimilis*; C. *Tabanus mularis*; D. *Tabanus sulcifrons*.


A. Hybomitra lasiophthalma
Percent Trapped
75
50
25
6-9AM 9AM-12 12-3PM 3-6PM 6-9PM


B. Tabanus subsimilis
Percent Trapped
75
50
25
6-9AM 9AM-12 12-3PM 3-6PM 6-9PM


C. Tabanus mularis
Percent Trapped
75
50
25
6-9AM 9AM-12 12-3PM 3-6PM 6-9PM

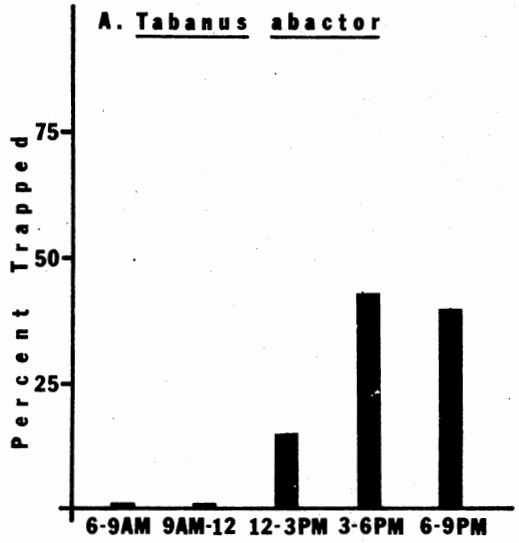

D. Tabanus sulcifrons
Percent Trapped
75
50
25
6-9AM 9AM-12 12-3PM 3-6PM 6-9PM

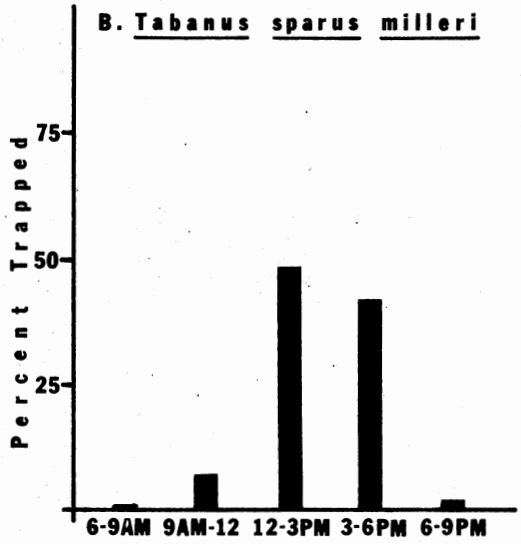
Only 16% of the total T. abactor were collected from 9 AM until 3 PM (Fig. 6). It's greatest activity occurred from 3 PM until 9 PM and they were equally abundant in both traps. Few T. abactor were observed attacking cattle, but this may be attributed to the much smaller population which was present in the area.

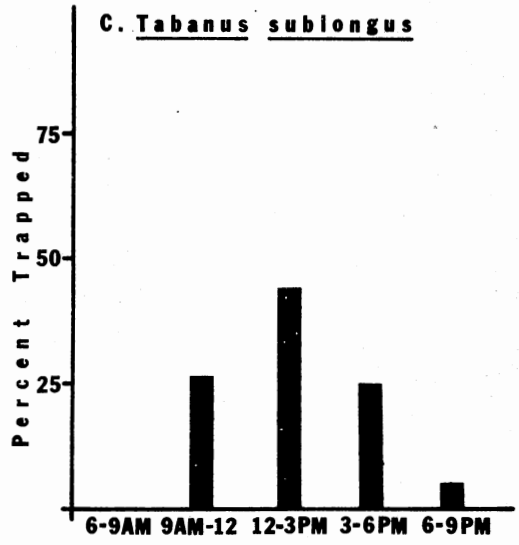
T. sparus milleri was captured primarily from noon until 6 PM in Poteau (Fig. 6), and only 10% were collected during other periods of the day. T. sublongus was most abundant in traps from 9 AM until 6 PM, with only 5% of the total collected after 6 PM (Fig. 6). T. abdominalis was trapped throughout the day, beginning with the 6 AM to 9 AM cycle, increasing in activity through the middle of the day, and then tapering off from 6 PM to 9 PM (Fig. 6). A total of 81% of this species was captured between 9 AM and 6 PM.

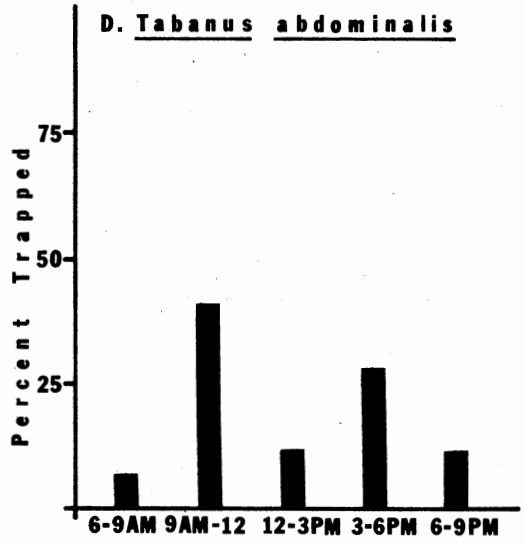
Thirty two species of Tabanidae were collected on the Kerr Foundation Ranch, Poteau, Oklahoma, a majority of which were Tabanus species. T. subsimilis, T. mularis and T. sulcifrons were the most abundant. A total of 9323 horse flies and deer flies were captured between April 13 and October 5. Diurnal activity of flies attacking cattle was similar to that observed in two Malaise trap collections.

Figure 6. Percentage of total tabanids collected by three hour trapping periods on the Kerr Foundation Ranch, Poteau, Oklahoma in 1979. A. Tabanus abactor; B. Tabanus sparus milleri; C. Tabanus sublongus; D. Tabanus abdominalis.


A. Tabanus abactor
Percent Trapped
75
50
25
6-9AM 9AM-12 12-3PM 3-6PM 6-9PM


B. Tabanus sparus milleri
Percent Trapped
75
50
25
6-9AM 9AM-12 12-3PM 3-6PM 6-9PM


C. Tabanus sublongus
Percent Trapped
75
50
25
6-9AM 9AM-12 12-3PM 3-6PM 6-9PM


D. Tabanus abdominalis
Percent Trapped
75
50
25
6-9AM 9AM-12 12-3PM 3-6PM 6-9PM

CHAPTER III

SEASONAL PARITY IN 4 TABANID SPECIES

Introduction

Parity in haematophagous Diptera is achieved after completing a gonotrophic, or egg laying, cycle. A fly that has already produced at least one batch of eggs is called parous; flies which have not laid any eggs are called nulliparous. The procedures for examining individual ovarioles to determine the number of gonotrophic cycles completed by a female, also referred to as physiological aging, were developed by Polovodova in Russia and described by Detinova (1962). When the number of ovarian cycles are known, or can be determined by examining ovarioles, females are described as uniparous, biparous, triparous, and so forth (Bertram 1962).

By determining the physiological age of numerous specimens of a population, it is possible to determine if a species is autogenous or anautogenous. Most haematophagous Diptera require a blood meal for completing the first ovarian cycle which is known as anautogenous reproduction. However, some species have developed the capacity to produce their first batch of eggs without taking a blood meal, which is referred to as autogeny. According to Spielman (1971) Roubaud first coined the term for the Culicidae, but it's usage has been expanded to include other haematophagous Diptera. If all the females dissected and examined when a population first emerges are nulliparous, then the

species is anautogenous; if a high percentage are parous at the start of the flight season, the species is autogenous.

Although well documented in some mosquito species, autogeny is apparently not as common in the Tabanidae (Barr 1974). Bailey (1948) speculated on the role of a blood meal in egg maturation in Tabanidae. Rockel (1969) was the first to report autogeny in a tabanid, C. fuliginosus, while Anderson (1971) reported autogeny in C. atlanticus and T. nigrovittatus. Gonotrophic cycles of numerous species were studied by Bosler and Hansens (1974), Magnarelli (1976), Morris and DeFoliart (1971), Saito (1967), Thomas (1972, 1973), and Troubridge and Davies (1975), but seldom did they report seeing more than the uniparous condition. Thompson et al. (1979c) suggested that this was because specimens were not held for an adequate period to permit contraction of the follicular relics. The purpose of this study was to determine if autogeny exists in tabanid species in north central Oklahoma, and to determine if distinct relics were apparent for each gonotrophic cycle.

Materials and Methods

Tabanidae were collected weekly with a modified Malaise trap (Roberts 1976) beginning with the first emergence of any species and continuing through September. Traps were baited with CO_2 gas which was continuously released from a 9.1 kg cylinder at the rate of 500 ml/min using a Matheson, Model 8 gas regulator. The rate of flow was calibrated with a Gilmont Model F-2060-A, shielded flowmeter. Traps were operated from 9 AM to 5 PM.

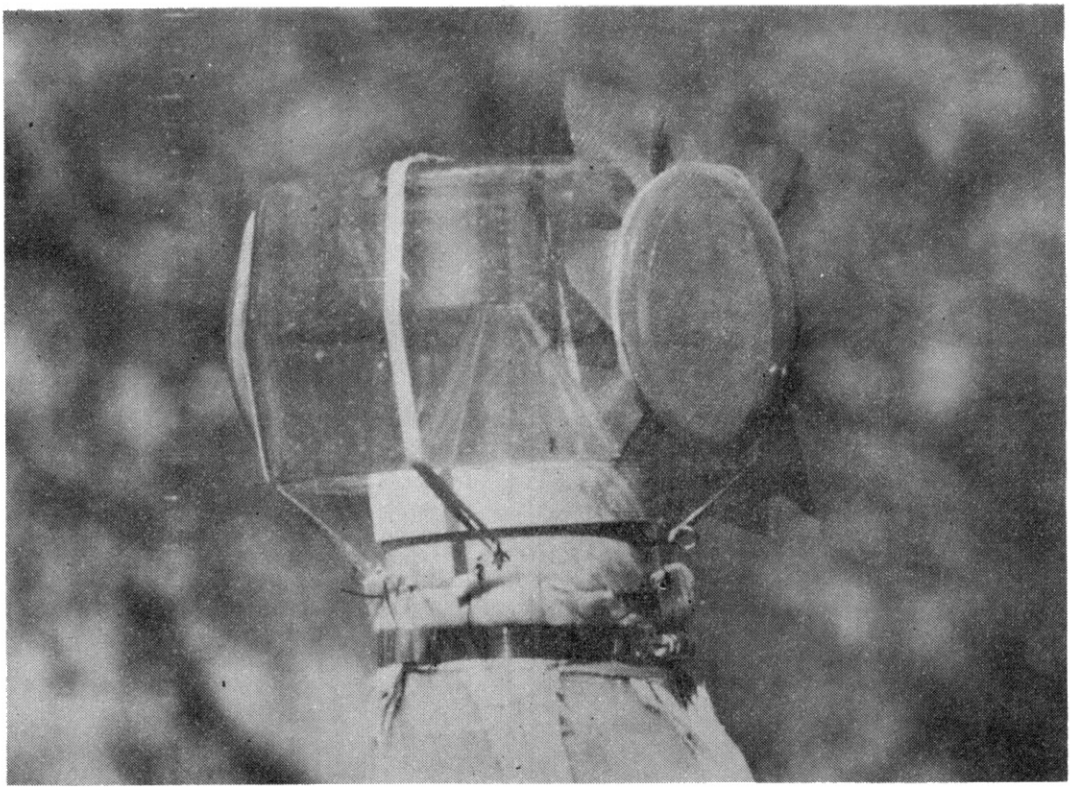
Collecting cannisters were made from 3.8 liter plastic fish bowls fitted with a jar lid and funnel from a one liter Nalgene® polypropylene

jar. The funnel was fitted inside the lid after which the two pieces were glued over a hole cut out of one side of the bowl so the funnel extended into the bowl (Fig. 7). The attached lid was placed over the top mounting ring of the Malaise trap and secured to it by rubber bands stretched over the bowl. A 15 cm diameter hole was cut in the bottom of the bowl and covered with 20 mesh per 2.54 cm Saran® screen. The top of the bowl was covered with a 15.24 cm square piece of 20 mesh screen held to the lip with a rubber band. This latter screen was easily removed to facilitate retrieval of flies from the cannister. When placed atop the trap, the bowl was on it's side so that the openings in the top and bottom let air circulate through the trap.

At periodic intervals, the collecting cannisters were removed and placed in an approximate 60 liter cooler containing freezer packs. The flies were returned to the laboratory where they were sorted and identified. Live females were maintained by species in groups of ten in 0.95 liter paper containers under a 16 hour light, 8 hour dark cycle at 26° C and 50-60% RH for five to seven days to permit adequate time for follicular debris to form dilatations, as suggested by Thompson et al. (1979c). Cartons were covered with a cloth mesh top and specimens were provided a 10% sucrose solution ad lib in cotton balls placed on the top of the container. Additionally, if a species was abundant, some specimens were either dissected immediately or frozen for later examination.

The initial capture of a large number of a species in the survey area was regarded as the beginning of the first emergence peak. At least 25 females of selected species were dissected during each successive week except in those instances when less than 25 specimens

Figure 7. Modified 3.8 liter plastic fish bowl with inverted funnel and mesh screen covers used for collecting live tabanids with a modified Malaise trap.

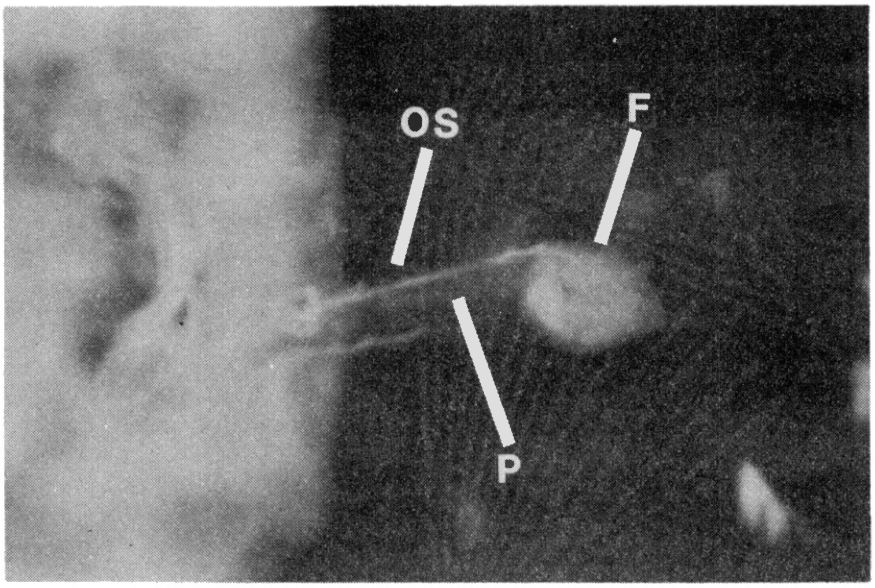


were collected in which case all were dissected. A technique similar to that of Thompson et al. (1979a) was used to remove the ovaries and separate ovarioles for examination. A minimum of six ovarioles per ovary were observed to determine physiological age using the techniques and criteria described by Detinova (1962). The same criteria have been used by Anderson (1971), Morris and DeFoliart (1971), Rockel (1969), Thomas (1972, 1973), Thompson et al. (1979a, 1979c), and Troubridge and Davies (1975) as a test for autogeny. Ovarioles of nulliparous flies had clear follicular tubes (Fig. 8), whereas parous flies had follicles in the sac stage (Fig. 9) or distinct relics in the pedicel (Fig. 10). When sac stage relics were observed, the fly was regarded only as a unipar since it could not be distinguished as having more than one relic. Flies with distinct relics or yellow bodies were grouped as unipars, bipars and tripars.

In addition to determining possible autogeny, physiological age grading was used to determine if females required more than one blood meal for completion of an ovarian cycle, if ovarioles contracted sufficiently to distinguish parity levels, and if tabanids actively sought additional blood meals for subsequent egg production. Blood engorged females were captured during imbibition from an immobilized cow by covering with an inverted, clear plastic cup as described by Tashiro and Schwardt (1949) and Hollander and Wright (1980b). Some specimens were immediately returned to the laboratory, while others were painted on the notum with Practra® enamel paints and released into a 6.1 X 8.5 X 2.5 meter natural Saran® screen cage located at the survey area. At two day intervals, a tethered cow was introduced into the cage and flies returning to feed were recaptured. All blood engorged females

Figure 8. Ovariole from a nulliparous tabanid female. OS - ovarian sheath; P - pedicel; F - follicle.

Figure 9. Ovariole from a parous tabanid female following oviposition. F - follicle; SS - sac stage relic in the pedicel.


OS
F
P

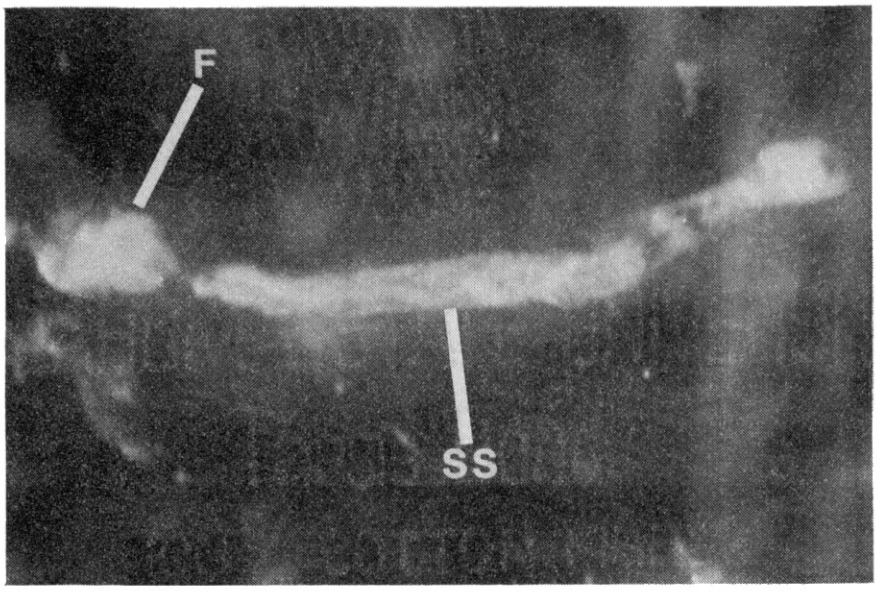
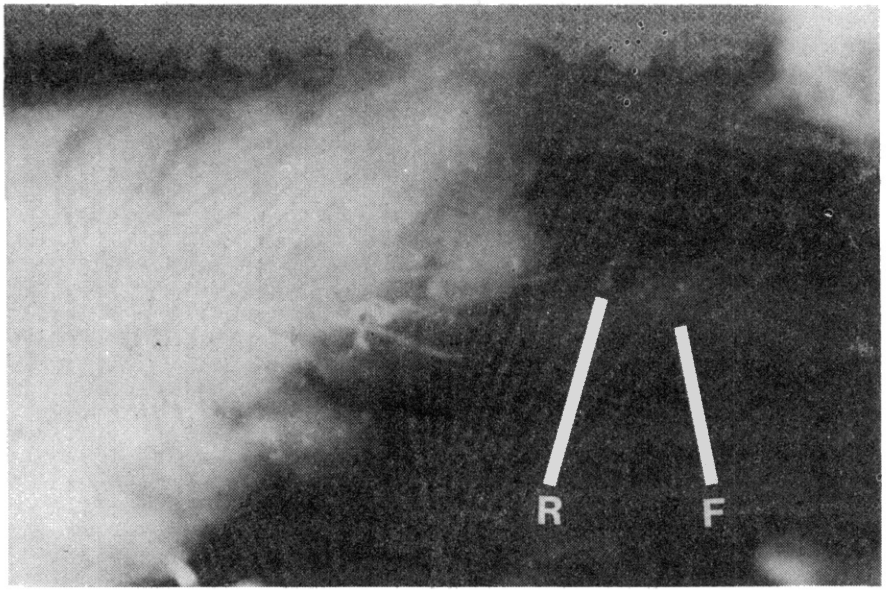

F
SS

Figure 10. Ovariole from a uniparous tabanid female. R - distinct relic; F - follicle.


R
F

taken to the laboratory were allowed to oviposit, and then held for five to seven days prior to examination for relics.

Results

Total tabanids collected in Malaise traps during 1980 exceeded 12,500. Table III shows the number of gonotrophic cycles completed by 1223 specimens of T. subsimilis, T. mularis, T. abactor and T. sulcifrons dissected from week one (May 11-17) through week 19 (September 14-20). Flies were held under insectary conditions for periods of five to seven days, therefore follicular debris had adequate time to form distinct relics in the pedicels. Uniparous, biparous and triparous females were observed in all four species. Nulliparous flies accounted for 28.5% of those dissected, while 42.2% were uniparous, 21.1% biparous and 4.1% triparous. Because a large number of nulliparous females were collected for each of the four species, it did not appear that any were autogenous. Additionally, since there were both parous and nulliparous females present for the duration of the season, continuous emergence was indicated in these species.

Many T. subsimilis collected between May 17 and June 14 were parous, although 100% parity did not occur until June 21, one to two weeks after the population peaked (Fig. 11). The percentage of the females that were parous was high for the rest of the trapping season, although there were always some nulliparous specimens present. Because T. subsimilis fed erratically near the feet of the cow, few blood engorged specimens were captured. Those released in the cage did not survive, but 12 engorged females were returned to the insectary where six oviposited and were dissected. Specimens that were collected as the flight season

TABLE III

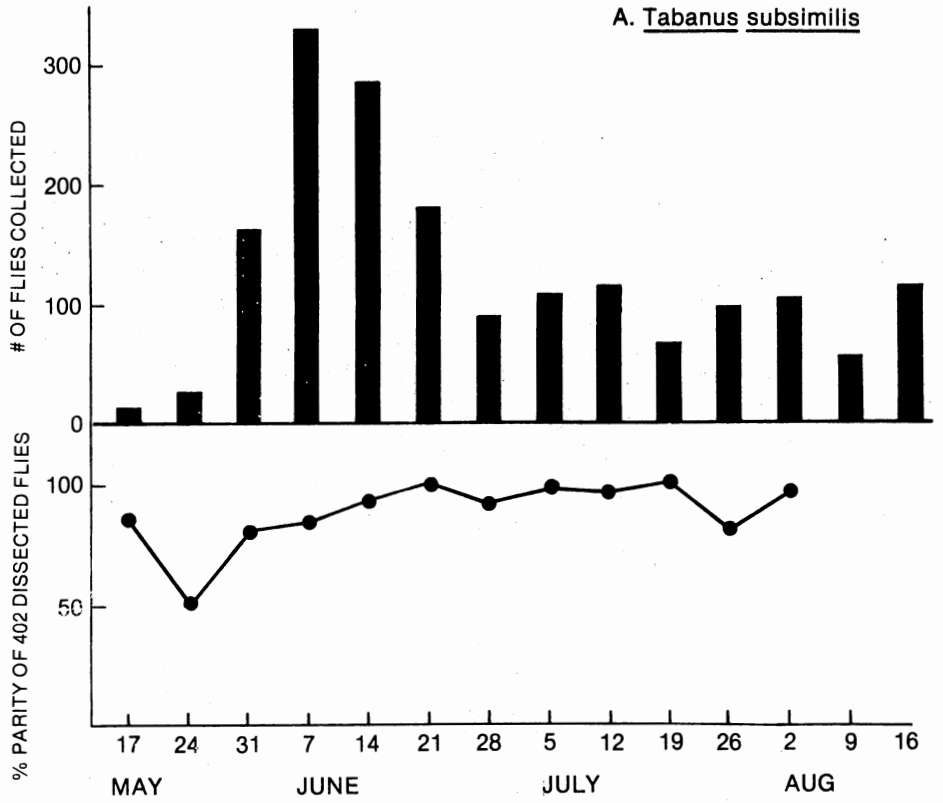
NUMBER (AND PERCENTAGE) OF GONOTROPHIC CYCLES OBSERVED IN FOUR TABANID SPECIES IN NORTH CENTRAL OKLAHOMA BETWEEN MAY 11 AND SEPTEMBER 20

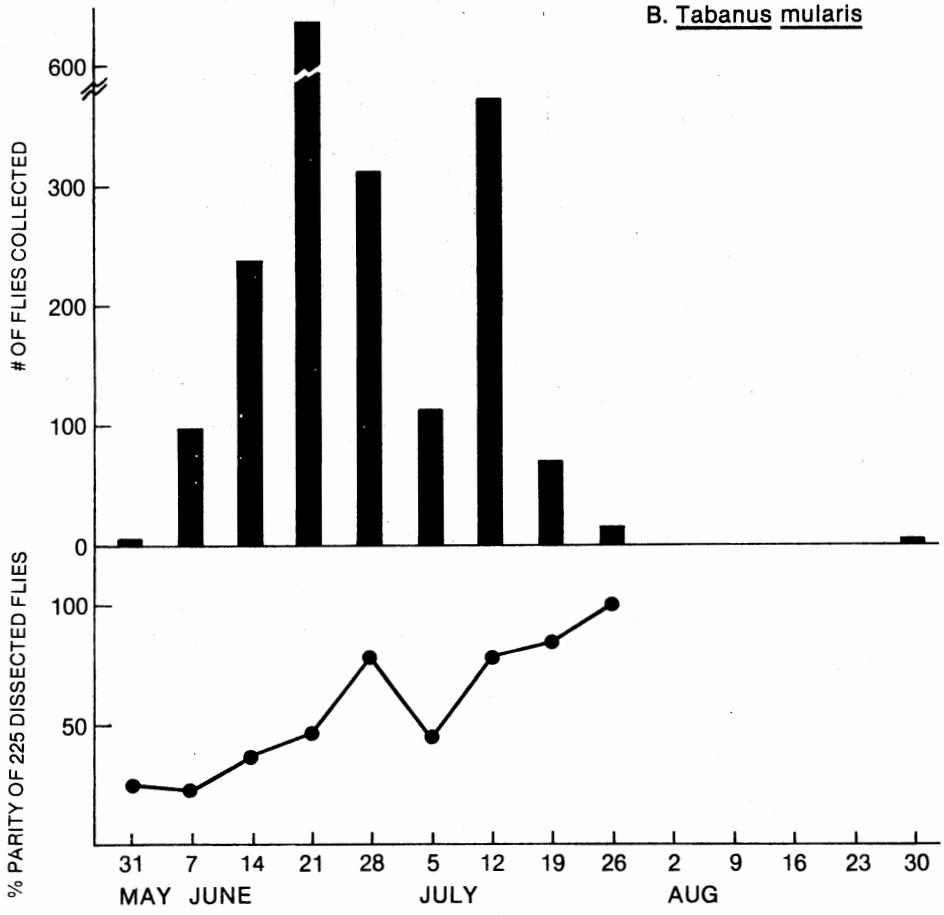
	Species															
	T. subsimilis				T. mularis				T. abactor				T. sulcifrons			
Week	N[a/]	U	B	T	N	U	B	T	N	U	B	T	N	U	B	T
1	1 (12.5)	7 (87.5)														
2	11 (50.0)	11 (50.0)														
3	8 (20.0)	30 (75.0)	2 (5.0)		3 (75.0)	1 (25.0)										
4	11 (15.7)	51 (72.9)	8 (11.4)		13 (76.5)	4 (23.5)			1 (100)							
5	6 (7.3)	48 (58.5)	27 (32.9)	1 (1.3)	38 (63.3)	22 (36.7)			29 (69.0)	11 (26.2)	2 (4.8)					
6		5 (83.3)	1 (16.7)		22 (53.7)	10 (24.4)	8 (19.5)	1 (2.4)	29 (50.0)	12 (20.7)	15 (25.9)	2 (3.4)	1 (100)			
7	1 (8.3)	9 (75.0)	2 (16.7)		6 (21.4)	10 (35.7)	11 (39.3)	1 (3.6)	33 (64.7)	11 (21.6)	4 (7.8)	3 (5.9)				
8	1 (2.6)	16 (42.1)	19 (50.0)	2 (5.3)	11 (55.0)	5 (25.0)	2 (10.0)	2 (10.0)	8 (26.7)	12 (40.0)	9 (30.0)	1 (3.3)	2 (100)			
9	1 (3.1)	22 (66.7)	9 (27.3)	1 (2.9)	4 (22.2)	11 (61.1)	3 (16.7)		5 (8.9)	46 (82.1)	4 (7.1)	1 (1.9)	6 (85.7)	1 (14.3)		
10		4 (33.3)	7 (58.3)	1 (8.4)	5 (15.2)	20 (60.6)	6 (18.2)	2 (6.0)		19 (76.0)	6 (24.0)		17 (94.4)	1 (5.6)		

11	3 (20.0)	9 (60.0)	2 (13.3)	1 (6.7)			2 (66.7)	1 (33.3)	21 (25.3)	54 (65.1)	8 (9.6)		21 (60.0)	13 (37.1)	1 (2.9)	
12	1 (3.8)	10 (38.5)	11 (42.3)	4 (15.4)					4 (10.3)	23 (59.0)	9 (23.1)	3 (7.6)	5 (11.6)	23 (53.5)	14 (32.6)	1 (2.3)
13													3 (16.7)	4 (22.2)	8 (44.4)	3 (16.7)
14													1 (6.3)	5 (31.3)	10 (62.4)	
15																
16	2 (15.4)		11 (84.6)		1 (100)						3 (50.0)	3 (50.0)	1 (14.3)	5 (71.4)	1 (14.3)	
17	2 (8.0)	2 (8.0)	10 (40.0)	11 (44.0)					4 (16.0)	7 (28.0)	12 (48.0)	2 (8.0)	5 (62.5)		3 (37.5)	
18																
19													2 (8.0)	12 (48.0)	8 (32.0)	3 (12.0)
TOTAL	48 (11.9)	224 (55.7)	109 (27.1)	21 (5.2)	103 (45.8)	83 (36.9)	32 (14.2)	7 (3.1)	134 (32.2)	145 (46.9)	72 (17.3)	15 (3.6)	64 (35.6)	64 (35.6)	45 (25.0)	7 (3.9)

[a] N - Nulliparous; U - Uniparous; B - Biparous; T - Triparous

Figure 11. Comparison of the total number of tabanids collected with the percent parity of dissected females in 1980. A. *Tabanus subsimilis*; B. *Tabanus mularis*.


A. Tabanus subsimilis
OF FLIES COLLECTED
300
200
100
0
% PARITY OF 402 DISSECTED FLIES
100
50
17 24 31 7 14 21 28 5 12 19 26 2 9 16
MAY
JUNE
JULY
AUG

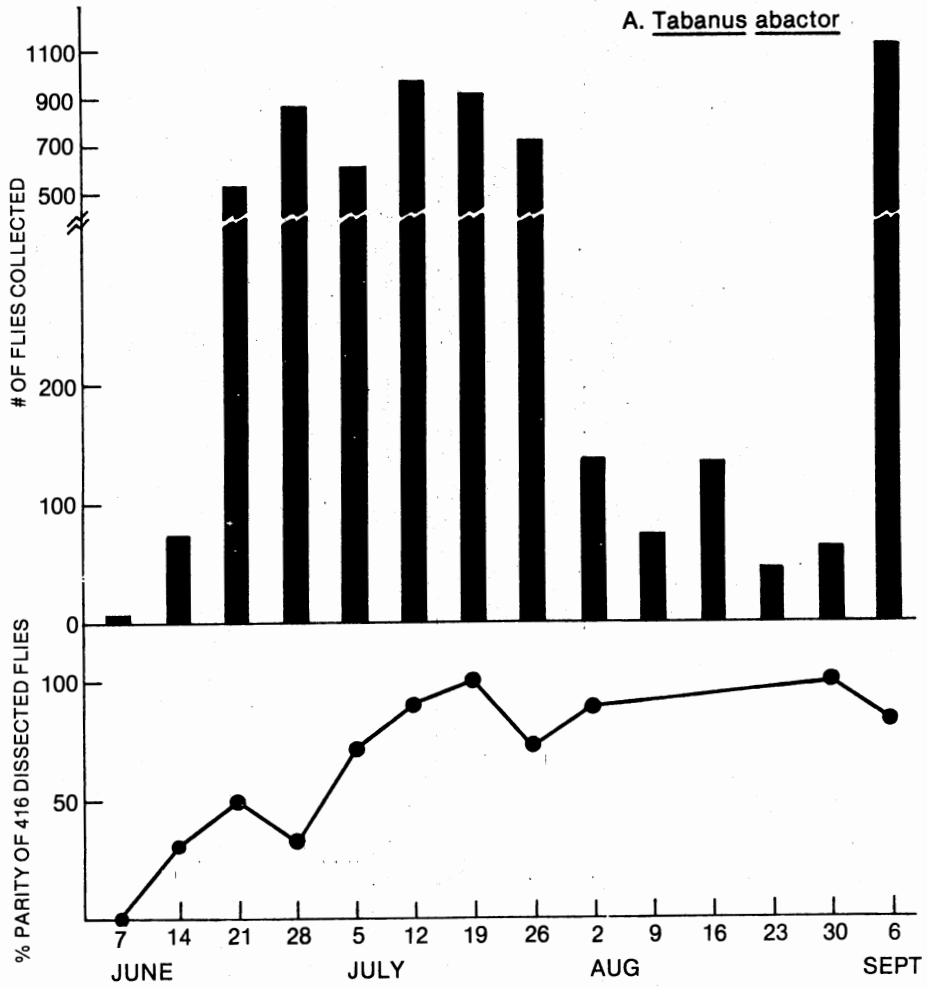

B. Tabanus mularis
OF FLIES COLLECTED
600
300
200
100
0
% PARITY OF 225 DISSECTED FLIES
100
50
31 7 14 21 28 5 12 19 26 2 9 16 23 30
MAY JUNE
JULY
AUG

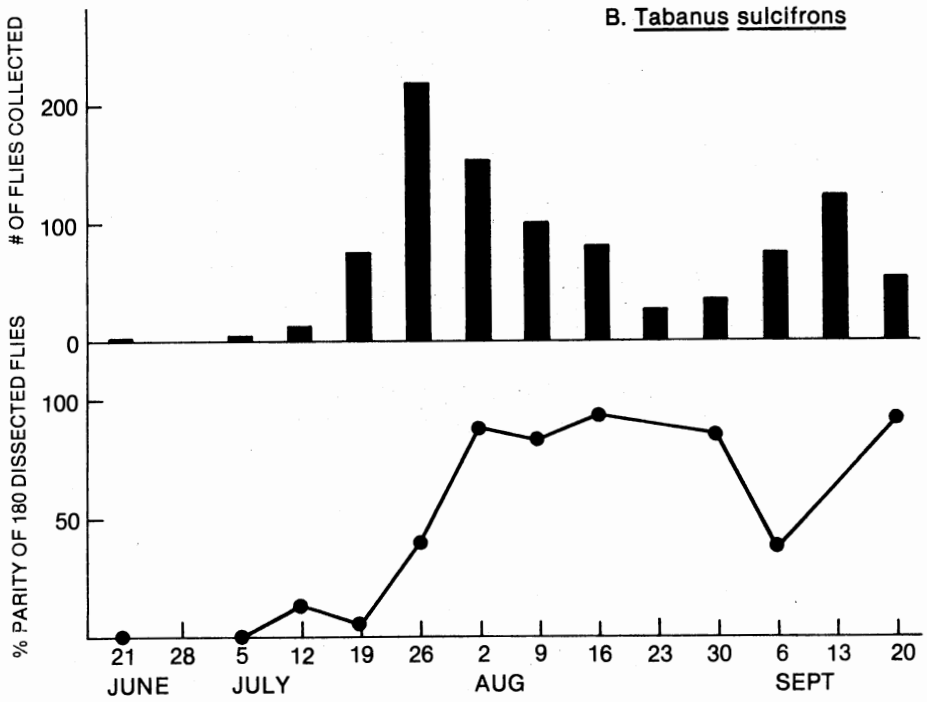
progressed and which were held for five to seven days, had dilatations indicating the uniparous, biparous and triparous condition.

The first T. mularis were collected on May 31. The number of parous females seen in 225 specimens was low initially and did not increase beyond 50% until one week after the population peaked on June 21 (Fig. 11). Less than 50% of the T. mularis dissected were parous when the population declined between June 28 and July 5. The percentage of specimens which were parous increased which indicated a bimodal emergence pattern. Fourteen days after the second population peak, 100% parity was seen (Fig. 11). In the laboratory, T. mularis appeared to undergo some adverse type of stress, as females were held for periods up to 28 days without ovipositing. Ovaries of these females contained fully developed follicles. In the caged environment, T. mularis females were observed coming to feed on the host in only two days, although some specimens took as long as five days. These specimens were held five to seven days in the laboratory and were observed to have distinct uniparous and biparous relics.

T. abactor appeared on June 7, but no parous individuals were collected until seven days later (Fig. 12). Less than 50% of the 416 females were parous during the first four weeks they were collected. The parity rate increased above 50% during the second week following the initial emergence peak of the week of June 21. Most specimens collected for the rest of the season were parous, although nulliparous females were still observed in the last collection on September 6. In the caged environment, T. abactor was recaptured taking a second blood meal in greater numbers than any other species, but this was probably due to the fact that more of this species was collected and initially

Figure 12. Comparison of the total number of tabanids collected with the percent parity of dissected females in 1980. A. *Tabanus abactor*; B. *Tabanus sulcifrons*.


A. Tabanus abactor
OF FLIES COLLECTED
1100
900
700
500
200
100
0
% PARITY OF 416 DISSECTED FLIES
100
50
7 14 21 28 5 12 19 26 2 9 16 23 30 6
JUNE
JULY
AUG
SEPT


B. Tabanus sulcifrons
OF FLIES COLLECTED
200
100
0
% PARITY OF 180 DISSECTED FLIES
100
50
21 28 5 12 19 26 2 9 16 23 30 6 13 20
JUNE
JULY
AUG
SEPT

introduced into the cage. Females were observed returning to the host in three to six days; usually after four days. Twelve of these specimens laid eggs in the insectary and when examined one week later had two distinct yellow bodies in the ovarioles which indicated a biparous condition.

A total of 180 T. sulcifrons were examined. A majority of specimens were nulliparous from the time they first emerged on June 21 until July 26 (Fig. 12). After the population peaked, the number of parous females increased to between 85-95%. One hundred percent parity was never observed in any samples of T. sulcifrons examined. In the cage, T. sulcifrons took from five to seven days to return to a host for a second blood meal, although only seven of 81 released survived.

Discussion

The completion of three gonotrophic cycles was observed in a total of 50 of 1223 females (4.1%) in four species, T. subsimilis, T. mularis, T. abactor and T. sulcifrons. This was more than has been reported for any other group of tabanid species. Thompson et al. (1979a) reported seeing one tripar among 410 T. nigrovittatus examined for relics, but others have not reported the triparous condition in parity studies. Thomas (1972) reported seeing 119 (9.4%) bipars from 1266 Hybomitra and Atylotus specimens examined in Alberta, while Troubridge and Davies (1975) found only 0.8% bipars in dissections of 20 tabanid species in Ontario. Magnarelli (1976) reported 55 bipars among 30 tabanid species examined in New York state, but Magnarelli and Anderson (1977) did not find any biparous relics in several salt marsh species studied. In each of these latter studies, dissections were performed, or flies

frozen, immediately after capture. Thompson et al. (1979c) emphasized the critical importance of holding females for several days after capture to insure complete ovarian contraction, and stated that age grading has little merit if the recognition of multiple gonotrophic cycles is obscured by the examination process. In this study, all specimens held for five to seven days before examination were found to contain distinct relics in their pedicels.

Thompson et al. (1979c) also suggested that changes in parity indicated anautogeny in a population. They observed that the change-over from nulliparous to predominantly parous females occurred over one week in H. lasiophthalma after which parous specimens were routinely collected. Similar conversion rates were observed in three of the species of Oklahoma tabanids, the exception being T. subsimilis. In that species, many females captured soon after emergence were parous, and this condition existed for the entire season. Utilizing relics as criteria for physiological aging, the population of tabanid species has been described as autogenous only when parity regularly exceeded 99% of females dissected (Anderson 1971; Magnarelli 1976; Morris and DeFoliart 1971; Rockel 1969; Thomas 1972, 1973). While the total number of parous T. subsimilis in this study was high, 48 or 402, or 11.9% were nulliparous and therefore, the population was considered to be anautogenous.

In a study of the seasonal changes in the physiological age of 15 tabanid species in southern Ontario, Troubridge and Davies (1975) suggested two hypotheses for high parous rates soon after emergence. They stated that different rates of seasonal increases in the proportion of parous females could be a function of emergence, in that species

having a rapid increase in parity rates were only present for a short period. This would not seem to be the case for T. subsimilis in Oklahoma since they were present for 12-16 weeks. An alternative hypothesis of Troubridge and Davies (1975) to explain the same data was that some species may undergo facultative autogeny which was interpreted to mean that only a protion of the original population was autogenous, but that most were anautogenous. In such a case, there would be a high level of parity when the population was at it's initial seasonal peak, and the number of biparous females would be significantly higher. In our study, the high ratio of parous females, including numerous bipars and tripars of T. subsimilis, may suggest facultative autogeny, but we felt that this species may have an alternate blood source rather than livestock which were our source of observations. Until we can confirm whether or not an alternate blood source was available, we do not feel we can classify the species as being autogenous.

In this study, the four tabanid species, T. subsimilis, T. mularis, T. abactor and T. sulcifrons, each had two different emergence peaks as was also reported by Hollander (1979). Although these peaks were evident, physiological aging of the four species showed the presence of nulliparous females throughout the season which indicated that there was continuous emergence.

Only one blood meal was required per ovarian cycle in the four species studies, although the time between imbibition and oviposition varied by species. Ovarian relics could be seen in these species when held for a suitable length of time following oviposition. Under natural conditions, subsequent refeeding of tabanids soon after oviposition initiated development of another ovarian follicle which

interfered with the ability to distinguish sac stage, or distinct relics of the previous parous cycle. This data confirms Thompson et al. (1979c) hypothesis that it is essential to hold collected specimens for several days to recognize individual relics. Additional studies are needed to examine blood feeding habits, dispersion and stages of follicle development in field populations of Tabanidae.

CHAPTER IV

PARAMETERS OF BLOOD MEAL DIGESTION AS RELATED TO OVARIAN DEVELOPMENT IN 5 TABANID SPECIES

Introduction

In the preceding chapter, it was reported that four species of Oklahoma Tabanidae were anautogenous for the first gonotrophic cycle. Therefore, these species are potentially good vectors of disease and important as pest species. More information is needed concerning the number of times a female can blood feed and produce eggs, the length of time between ovarian cycles, and the life span of these species. Oviposition has been studied in tabanids to determine egg laying characteristics and viability (Magnarelli and Stoffolano 1980; Roberts 1966; Schwardt 1936; Thompson et al. 1979b). Research has also been done using various marking techniques to study flight ranges and dispersal of different species (Beesley and Crewe 1963; Bennett and Smith 1968; Harlan and Roberts 1976; Sheppard et al. 1973; Thornhill and Hays 1972). Mark-recapture techniques, however, have not been utilized to aid in the study of the parameters of blood meal digestion as related to ovarian development in horse flies.

The objective of this study was to examine reproductive activity in five major pest species. This included laboratory determinations of the time required between blood engorgement and egg maturation in T. subsimilis, T. mularis, T. sulcifrons, T. abactor and T. atratus.

Field observations using mark-recapture techniques were used to study the activity of these five species in an attempt to determine the number of days needed for digestion of a blood meal, oviposition and the resumption of host seeking activity.

Materials and Methods

The survey area used in this study was a large, rangeland pasture located in Payne Co., Oklahoma consisting of approximately one third post oak and scrub oak thicket, and bordered by Lake Carl Blackwell to the north and east. Tabanids in the area have been surveyed for three years and a large population was known to exist (Hollander 1979; Wright personal communication). Female tabanids were attracted to the survey site by a Jersey cow tethered between two poles on the fringe of a large, shaded thicket. Blood seeking flies were allowed to land free choice and feed to repletion. No effort was made to restrict leg or tail movement by the cow, nor were flies manipulated to enhance feeding.

Fully engorged flies were taken from the host by covering with a clear, inverted plastic cup as described by Hollander and Wright (1980b). These were placed in a refrigerated ice chest, returned to the laboratory for sorting, and sample sizes of from two to five adults of each species were held in 0.47 liter paper cartons with cloth mesh covers. Flies were fed 10% sucrose ad lib in cotton balls placed on top of the mesh covers.

Twenty four hours after capture, specimens of the five species were examined. Females were impaled dorsally against a wax filled watch glass and the abdominal cavity exposed by making a longitudinal slit along the abdominal sternites using eye scissors. Measurements

were made of the length and width of the engorged midgut, and observations made on the condition and color of gut contents. The ovaries were examined under 10-70 magnification and the stage of follicular development was determined using the following classification as described by Christopher (1911) and Mer (1936): stage I, no yolk in the oocyte; stage II, yolk deposition begins; stage III, yolk almost completely conceals the oocyte; stage IV, follicle begins to lengthen; and stage V, exochorionic structures appear on the surface of the maturing egg. Each stage is further divided into an early and late phase, generally characterized by subtle changes of the developing follicle. For example, in early stage III yolk granules occupy two thirds of the follicle whereas this increases to three fourths of the follicle by late stage III. Specimens of each species were examined daily for seven days postfeeding or until all females had stage V follicles.

In order to study the time of blood meal digestion under field conditions, specimens were marked while feeding on the tethered cow with Practra® enamel paints (Service 1976; Thornhill and Hays 1972) and then released. The first specimens were marked on July 11 and were continuously marked from 9 AM to 5 PM daily on July 21 to July 25. Painted flies were recaptured over a 30 day period from July 11 to August 11 with CO_2 baited Malaise traps (Roberts 1976) and on the Jersey cow. Three traps were erected in a triangular pattern at distances of 183, 259 and 366 meters from the release site and baited using CO_2 gas released at 250 ml/min. Collection cannisters each contained 2.54 cm squares of dichlorvos resin for rapid knockdown of the flies. The cow was tethered at the release site between 9 AM and 5 PM every day until marked flies were no longer observed returning to feed.

In the laboratory, unmarked flies were sorted to species and counted. Marked flies were individually held in 0.47 liter paper cartons and fed sucrose ad lib under a 16 hour light, 8 hour dark cycle at 26° C and 50-60% RH. After five to seven days, they were dissected to examine ovarian follicle development using techniques described by Detinova (1962).

Results

A total of 185 blood engorged females of five species were collected from a cow and held in the laboratory for daily examination. Eighteen blood engorged T. subsimilis were collected and dissected, due to the small August population of the species and because of the difficulty in collecting blood engorged specimens from their preferred feeding site on the feet and legs. T. subsimilis rapidly digested a blood meal within 48 hours (Table IV). Ovarian follicles had usually begun differentiating on day one, and all were in late stage III two days after a blood meal (Table V). Fully mature eggs were found in all T. subsimilis females dissected three days postfeeding. Twenty two females of the species were marked for release to the field, but none were observed returning to the host to refeed.

T. mularis developed mature eggs within four days from the time of blood engorgement (Table V), but follicle differentiation took longer to become apparent. The blood meal was generally small and the midgut dimensions rapidly decreased (Table IV). Many specimens were observed with late stage II follicles within 24-48 hours, but it was between two and four days postfeeding when rapid follicle differentiation was most apparent. T. mularis was nearing the end of its flight season,

TABLE IV

CHANGES IN MEAN MIDGUT MEASUREMENTS (WIDTH X LENGTH ± SD IN CM) OF 5 TABANID SPECIES OVER A ONE WEEK PERIOD FOLLOWING BLOOD ENGORGEMENT. SAMPLE SIZE SHOWN IN ( )

Days Post Feeding	Species				
	T. subsimilis	T. mularis	T. atratus	T. sulcifrons	T. abactor
1[a/]	0.28±.04 x 0.53±.04[b/] (2)	0.32±.06 x 0.56±.08 (7)	0.73±.15 x 1.21±.13 (3)	0.67±.05 x 0.97±.10 (4)	0.45±.04 x 0.76±.10 (9)
2	0.14±.06 x 0.43±.06 (3)	0.24±.03 x 0.43±.09 (5)	0.47±.06 x 0.73±.09 (4)	0.54±.03 x 0.86±.04 (5)	0.36±.05 x 0.68±.12 (10)
3	0.10±.01 x 0.28±.03 (4)	0.21±.03 x 0.30±.02 (5)	0.34±.05 x 0.71±.02 (4)	0.44±.06 x 0.93±.22 (6)	0.27±.05 x 0.58±.08 (10)
4	none (5)	0.19±.03 x 0.35±.04 (7)	0.30±.01 x 0.50±.01 (6)	0.38±.08 x 0.82±.06 (6)	0.19±.06 x 0.52±.10 (10)
5	none (2)	0.16±.02 x 0.22±.06 (5)	none (3)	0.27±.08 x 0.53±.08 (8)	0.15±.05 x 0.46±.12 (10)
6	---	0.21±.05 x 0.30±.01 (2)	none (2)	0.30±.06 x 0.59±.09 (8)	none (6)
7	---	none (5)	---	none (6)	---

[a/] First measurement is the engorged size at 24 hours postfeeding

[b/] All measurements based on guts which had remnants of blood

TABLE V

STAGES OF OVARIAN FOLLICLE DEVELOPMENT IN 5 TABANID SPECIES OVER A ONE WEEK PERIOD FOLLOWING BLOOD ENGORGEMENT. SAMPLE SIZE SHOWN IN ( )

Days Post Feeding	Species				
	T. subsimilis	T. mularis	T. atratus	T. sulcifrons	T. abactor
0	- - - - - - - - - - - All specimens blood engorged on day 0 - - - - - - - - - - -				
1	E-III to L-III[a/] (2)	E-II to L-II (7)	E-II to E-IV (4)	L-I to E-II (6)	E-II (10)
2	L-III to V (4)	L-II to E-IV (7)	L-IV to V (4)	L-III to L-IV (6)	L-III to L-IV (5)
3	Mature (4)	V (7)	Mature (4)	L-IV (6)	L-IV to V (10)
4	Mature (4)	Mature (7)	Mature (4)	V (11)	Mature (10)
5	Mature (2)	Mature (7)	Mature (3)	V (11)	Mature (10)
6	Mature (2)	Mature (7)	Mature (3)	Mature (11)	Mature (10)

[a/] E denotes early phase in the stage of ovarian development, whereas L denotes the late phase; E-II is often referred to as the resting stage of ovarian development

and as such, only 28 were marked and released. No specimens were recaptured.

Only 61 T. atratus were collected in this study, but the females were readily taken in plastic cups, or marked for release after blood engorgement. This species imbibed large quantities of blood (Hollander and Wright 1980b) which was slowly digested over a four day period (Table IV). The blood appeared stiff or clot-like when examined in the midgut. Ovarian follicles of T. atratus showed rapid differentiation, reaching late stage IV to stage V in two days, and most were mature in three days (Table V). Five (12.8%) of 39 marked females were recaptured attempting to take a second blood meal after only two days. Two were dissected and found to contain sac stage relics, indicating recent oviposition. Two others were held for seven days and when dissected had two distinct yellow bodies in the pedicels of each ovariole examined indicating a biparous condition. The fifth T. atratus was permitted to complete a second blood meal prior to capture, and when examined three days postfeeding, the ovaries were fully gravid. This demonstrated the demand for immediate host seeking activity by the species following the completion of an ovarian cycle.

T. sulcifrons required the longest time for eggs to mature following a blood meal. Follicles reached late stage III or early stage IV by two days, but required another two days to fully lengthen and for exochorionic structures to appear (Table V). When examined after five days, most contained fully mature eggs, whereas flies held for six days were always found to be fully gravid. Two of 121 marked female T. sulcifrons were recaptured in the 30 days of the survey. They were taken in a Malaise trap 1073 meters from the release point on days five

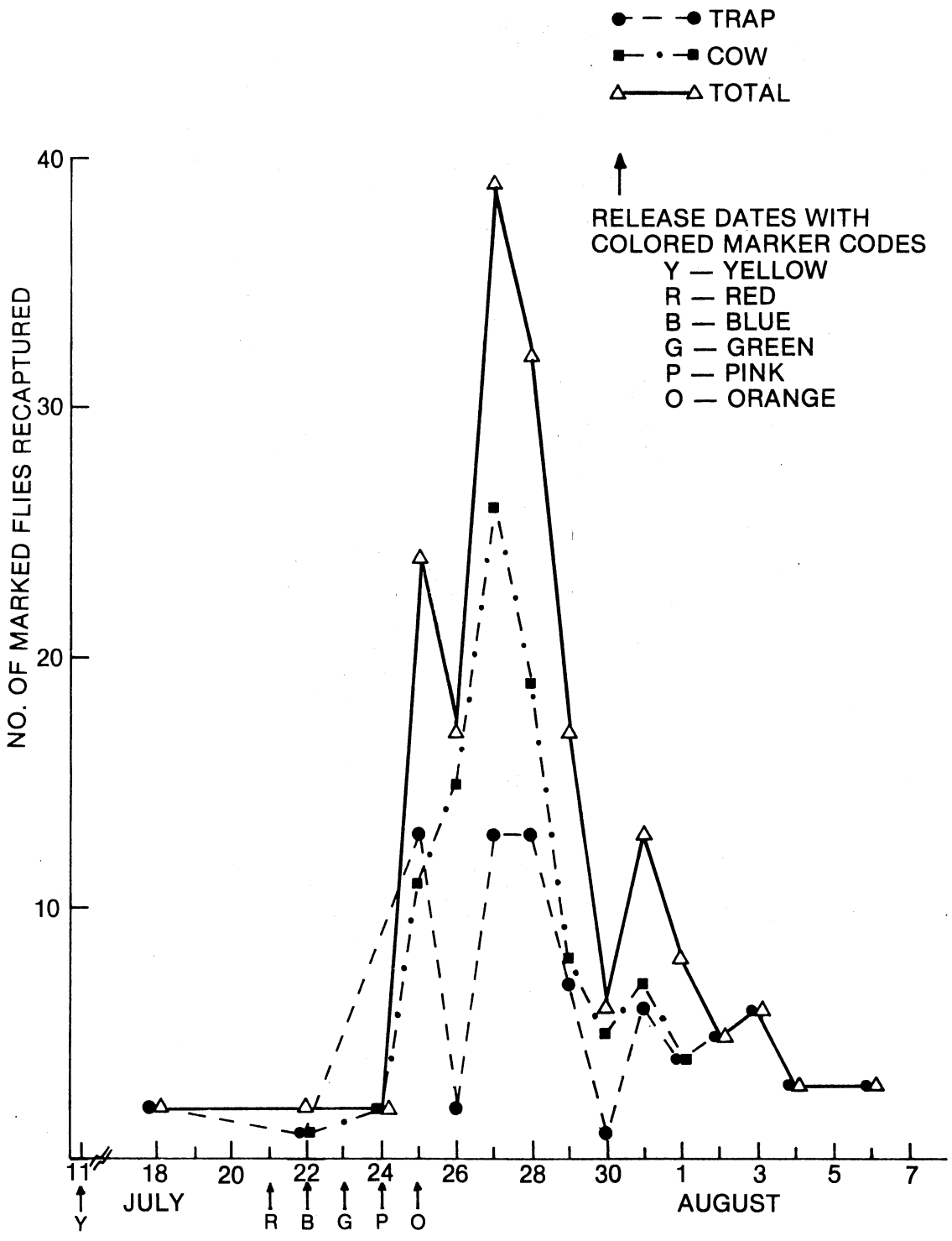
and six postfeeding. After feeding, they were generally observed flying uninterrupted towards open areas and even though they imbibe large amounts of blood, they remained strong fliers.

T. abactor developed mature eggs in four days, similar to T. mularis (Table V). This species imbibed large quantities of blood which was still apparent in the midgut after ovaries had become distended with ripening eggs (Table IV). All of the blood engorged T. abactor captured and held for a minimum of four days contained fully mature eggs.

A total of 1700 T. abactor were marked and released after taking blood meals with 1450 of these marked during five consecutive days. Many of the blood engorged specimens flew short distances from the host before landing and resting in the foliage of trees or on range grasses. One hundred and eighty two of these, or 10.7% of the total T. abactor released, were recaptured over a 30 day period (Fig. 13). One hundred and three of the marked flies were recaptured while seeking a second blood meal from the host in the same area where the first feeding occurred. Seventy nine marked specimens were captured in Malaise traps, of which 77 were evenly distributed among the three nearest traps, located 183, 259 and 366 meters from the release point. Host seeking activity by T. abactor increased on the fourth day after the July 21 release date, and most (39) were collected three days after the July 25 release date (Fig. 13). Marked flies were no longer taken on the cow or in traps after 14 days postfeeding although Malaise traps were continuously operated for an additional week.

The number and percentage of marked T. abactor females recaptured on consecutive days increased greatly on the cow on the third day after

Figure 13. Number of marked Tabanus abactor recaptured using two methods over a 27 day period in 1980.

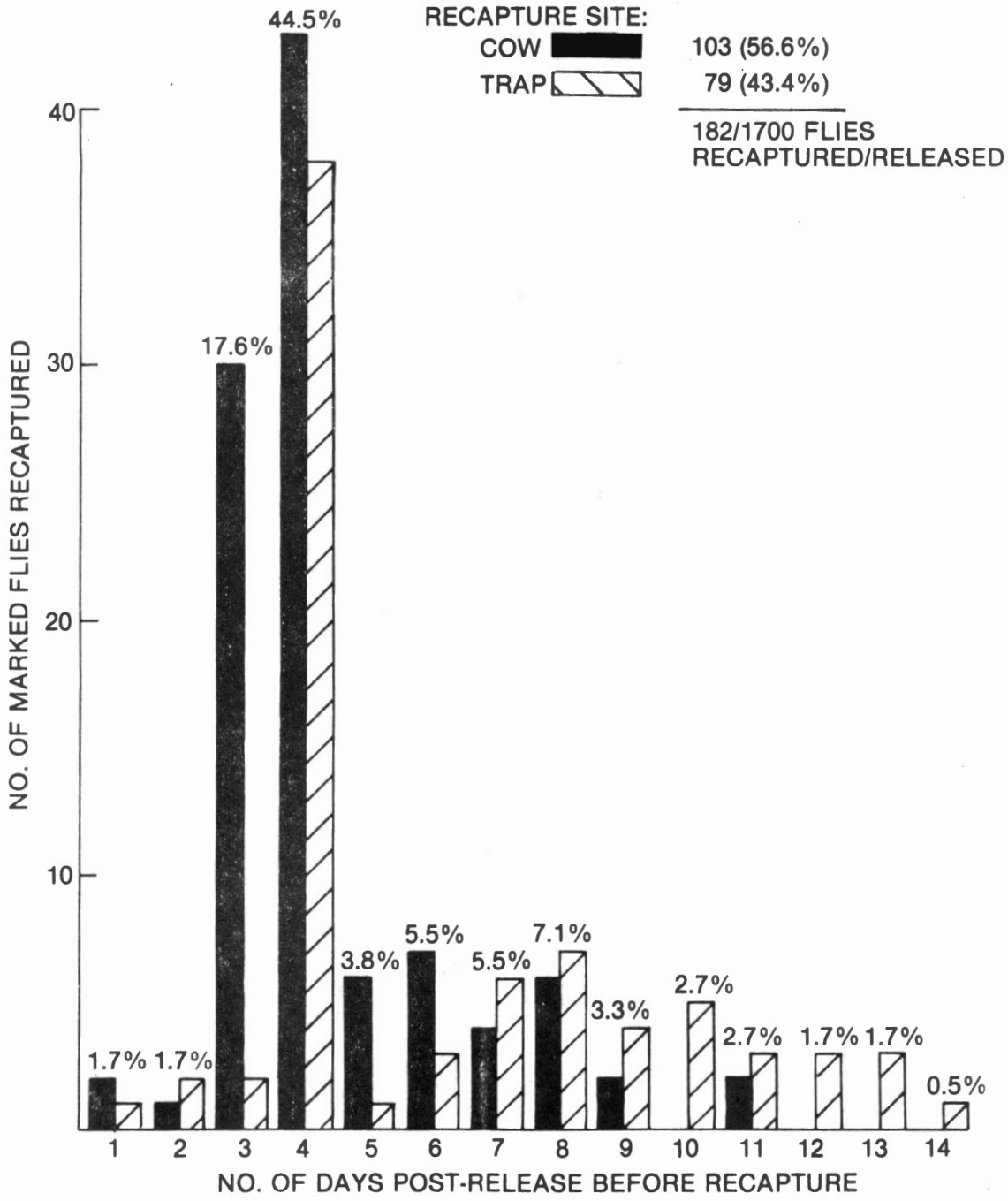

TRAP
COW
TOTAL
RELEASE DATES WITH COLORED MARKER CODES
Y — YELLOW
R — RED
B — BLUE
G — GREEN
P — PINK
O — ORANGE
NO. OF MARKED FLIES RECAPTURED
40
30
20
10
11
18
20
22
24
26
28
30
1
3
5
7
JULY
AUGUST
Y
R
B
G
P
O

release and on both the cow and in traps on the fourth day (Fig. 14). The number of marked flies recaptured decreased by the fifth day after release and only 34.5% of the total were recaptured between days five and twelve. All marked T. abactor females which were examined in the laboratory were parous, that is, had already completed at least one gonotrophic cycle, and their follicles were in the resting stage (E-II). Marked T. abactor females coming to the cow which were allowed to feed a second time, were all found to contain fully mature eggs after being held in the laboratory for four days.

Discussion

A marking technique using Practra® enamel paints applied to the notum of female horse flies during feeding was demonstrated to be as successful for the study of reproduction in certain Tabanidae as for the longevity and dispersal studies done by other authors (Harlan and Roberts 1976; Sheppard et al. 1973). More extensive trapping within a survey area, as well as more complete marking of flies for each 24 hour period is regarded as desirable for future efforts. It would appear that the brief period of time between ovarian cycles demonstrated herein may indicate a need for increased concern regarding the epidemiologic role of Tabanidae, especially in the absence of autogenous ovarian follicle development. The rapid digestion of blood meals by the species studied was directly correlated to the likewise rapid maturation of ovarian follicles. Follicle differentiation became apparent in five tabanid species within 24-48 hours after imbibition of a blood meal and the development of mature eggs occurred in some species in two, three and four days when observed in the laboratory and under field conditions.

Figure 14. Number and percentage of marked *Tabanus abactor* females recaptured using two methods at one day intervals in 1980.


RECAPTURE SITE:
COW
TRAP
103 (56.6%)
79 (43.4%)
182/1700 FLIES
RECAPTURED/RELEASED
NO. OF MARKED FLIES RECAPTURED
40
30
20
10
1.7%
1.7%
17.6%
44.5%
3.8%
5.5%
5.5%
7.1%
3.3%
2.7%
2.7%
1.7%
1.7%
0.5%
1
2
3
4
5
6
7
8
9
10
11
12
13
14
NO. OF DAYS POST-RELEASE BEFORE RECAPTURE

The mark-recapture of 182 T. abactor in this study indicated that tabanids can take repeated blood meals for egg laying, but the maximum period for survival was 14 days. Life spans of between 14 and 23 days were also reported by Harlan and Roberts (1976) and Thornhill and Hays (1972) in T. fulvulus Wiedemann, T. lineola, T. melanocerus, T. nigripes, T. pallidescens Philip and T. petiolatus Hine. For species with long periods of flight activity, this suggests that individuals must be continuously emerging, and this was also demonstrated in the parity study in Chapter III.

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