MALE-FEMALE INTERACTION AMONG DIFFERENT GEOGRAPHIC STRAINS OF THE GULF COAST TICK, *AMBLYOMMA maculatum* KOCH

A Thesis

by

SARAH B. SLEEBA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2005

Major Subject: Entomology

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Approved as to style and content by:

Pete D. Teel (Chair of Committee)	Michael T. Longnecker (Member)
Darrel E. Bay (Member)	
Kevin M. Heinz (Head of Department)	

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ABSTRACT

Male-Female Interaction among Different Geographic Strains of the Gulf Coast Tick, *Amblyomma maculatum* Koch. (May 2005)

Sarah B. Sleeba, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Pete D. Teel

The overall goal of this research was to examine the interactions of adult *Amblyomma maculatum* Koch, the Gulf Coast tick, with respect to their utilization of hosts and to male-female cross strain interaction. Historical data along with two Petri dish experiments were used to understand male-female interaction in the field, and to determine if the aggregation attachment pheromone (AAP) produced by fed males of varying strains is attractive to geographic specific strains of unfed female ticks.

It was hypothesized that questing female Gulf Coast ticks are attracted to fed males and can discriminate between grazing cattle with fed males and those without. Archival control data from ear tag studies conducted in 1985, 1987, and 1991 were analyzed to better understand female Gulf Coast tick behavior in the field relative to fed male tick presence. Females were found primarily on hosts with an abundance of male ticks, leading one to conclude that female ticks are attracted to hosts infested with male ticks. It was also discovered that females were more likely to be found on a host as the number of males on a host increased. A female's ability to detect hosts parasitized by males likely allows them to feed and mate on-host in a fairly limited period of time.

A Petri dish bioassay was used to evaluate female preference to varying geographic strains of fed males. One experiment was designed to determine if a female preferred fed males from her geographically specific strain over other males. A second experiment evaluated female response to a non-specific male in the absence of her geographically specific male. While female responses to fed males regardless of strain were higher than to unfed male control ticks, no statistical differences in female response could be determined. The Petri dish bioassay was determined to be inadequate to test female preference over several populations of pheromone producing males, and a more intensive procedure was proposed.

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I would like to give my sincere gratitude and thanks to my committee chair, Dr. Pete Teel. You have encouraged me, broaden my horizons and have given me the tools to be the best I can be. During my time here your patience and understanding made everything a little easier.

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To all of my friends, you know who you are, I want to thank you for keeping me sane. Sometimes all you need is laughter to solve the most complex of problems.

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CHAPTER I

INTRODUCTION

In the early Paleozoic era, ticks survived as obligate parasites of reptiles. Reptiles evolved over time, and as birds and mammals became the dominate vertebrates, ticks evolved to follow suit. Modern ticks have evolved behavioral and structural adaptations to survive and multiply. *Rhipicephalis, Boophilus,* and *Haemaphysalis* species have developed broad palps (unlike their Paleozoic ancestors) which allow them to move quickly through hair and feathers in order to find adequate attachment sites and mates. *Ixodes ricinus* and *I. persulcatus,* parasites of cattle and other ungulates, will delay their drop off until cattle are out to pasture, an ideal place for a female to drop and find a place to oviposite (Hoogstral 1978). It is proposed that female Gulf Coast ticks, *Amblyomma maculatum Koch*, have evolved a way to not only locate and attach to viable hosts, but viable hosts with male Gulf Coast ticks already attached.

The Gulf Coast tick, *Amblyomma maculatum* Koch, is an ectoparasite found along the Gulf Coast from Mexico to Florida and up the Atlantic Coast to North Carolina, as well as inland in Oklahoma and Texas (Williams 2002). In these regions it is recognized as a major economic pest of livestock (Drummond and Whetstone 1970).

Gulf Coast ticks are serious livestock pests. Gulf Coast ticks are a three host tick. The immature of this species feed on birds and small mammals, the adults, however, feed primarily on ruminants. As a result of Gulf Coast tick infestation, livestock often

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This thesis follows the style and format of the Journal of Medical Entomology.

experience decreased body weight, irritation and/or secondary infection (Riley et al. 1995). There has been added concern regarding the possible impact of Gulf Coast ticks and the ability to control them. This is due primarily to the fact that researchers have recently discovered that Gulf Coast ticks can vector heartwater (Mahan et al. 1999). Heartwater is a highly virulent infectious disease of ruminants caused by the pathogen *Ehrlichia ruminantum*. The mortality rate for infected cattle can be up to 60%. It is currently not found in the United States, however, it can be found as close as the Caribbean (Deem et al. 1996). If Heartwater was introduced to the United States, the pathogen could be quickly spread by Gulf Coast ticks, resulting in great economic loss (Bram et al. 2002, Deem et al. 1996). Concern over our ability to control these ticks has led researchers to look for other possible ways to control them.

The Gulf Coast tick is among the few species of *Amblyomma* ticks that have been discovered to produce aggregation attachment pheromones or AAP (Rechav et al. 2000) Aggregation Attachment Pheromones are chemicals produced by one group of organism (eg. fed male ticks) to alter another group's (eg. female ticks) behavior by attracting them to a particular site on a host and encouraging attachment (Sonenshine et al. 1982) These pheromones are produced by fed or feeding males (Sonenshine et al. 1982) Early work (Gladney 1970) indicated that under laboratory conditions female Gulf Coast ticks rarely attach to hosts in the absence of males. Gladney et al. (1974) later found that female Gulf Coast ticks attached to areas on cattle treated with an extract from fed male Gulf Coast ticks. Using a Petri dish bioassay technique, Rechav et al. (2000) demonstrated that unfed females were attracted to fed males. Gladney et al. (1974) concluded that these AAP may

have important applications in the monitoring of Gulf Coast ticks, however, such applications have yet to be explored for this species.

There are two potential applications of AAP, on and off host. For on host control they can be incorporated into pesticide impregnated tags, collars and bands to aid in tick control and population suppression (Allan et al. 1998, Norval et al. 1996, Gladney et al.1974). For off host control, they could be added to Carbon Dioxide (CO_2) traps to aid in monitoring these ticks or in combination with pesticides to also aid in population suppression. Pesticide impregnated ear tags are currently the primary tick control tactic used on cattle ranches. If AAP were added to ear tags it would attract ticks close to the pesticide, resulting in an increase in exposure to the pesticides (Gladney et al.1974, Allan et al. 1998), thus increasing ear tag efficacy and lessening the chance of spreading disease causing pathogens by free living ticks. The most commonly used off host tick collection method used to date is CO_2 traps. In order to locate a host most ticks use their chemo-sensory organs located on their fore tarsi to detect CO_2 produced by potential hosts (Rechav et al. 1977.) A CO_2 trap takes advantage of this behavior by using dry ice as a source of CO_2 to attract ticks into a sticky trap. Unfortunately, Gulf Coast ticks are rarely attracted to these traps (Bengaly 1987). If AAP were added to these traps their level of attraction to Gulf Coast ticks may be greatly increased.

There are currently three known unique geographic strain of Gulf Coast ticks. A geographic strain is differentiated by differences in seasonal activity and genetic variation. Geographic strains of Gulf Coast ticks often exhibit different seasonal activity patterns. Texas Gulf Coast strain adults reach peak activity in September, whereas Oklahoma and Kansas strains reach peak adult activity in April-May. Among the three

geographic strains of Gulf Coast ticks, there is significant variation in seven alleles of the 12s r DNA gene, indicating greater genetic diversity in the inland strains. Recently, a difference in profiles of volatile organic compounds believed to be associated with the AAP from fed males suggests differences among geographic strains in pheromone communication (Kim, 2004).

The overall goal of this research was to examine the interactions of adult *Amblyomma maculatum*, the Gulf Coast tick, with respect to their utilization of hosts and to male-female pheromone communication. The objective was approached in two ways; (1) evaluate the male-female dynamics of adult attachment under field conditions; (2) up determine whether female Gulf Coast ticks are equally attracted to fed males of different geographic strains.

CHAPTER II

FEMALE GULF COAST TICK ATTRACTION BASED UPON MALE PRESENCE ON PASTURED CATTLE

Female Gulf Coast ticks, like other ixodid ticks, have developed a strategy to improve their success in host finding, bloodmeal acquisition, and mating. Although they appear to be attracted to hosts infested with males little is currently known about tick-host attraction and attachment in the field. Fleetwood (1985) discovered that overwintering Gulf Coast tick males in a simulated microhabitat became active and quested at the base of the vegetations prior to female ticks. Gladney et al. (1971) showed that female Gulf Coast ticks once on a host preferred to attach to the cow in proximity to fed males. Gladney later discovered that this may be due to an aggregation pheromone produced by fed male Gulf Coast ticks (Gladney et al. 1974, Sonenshine et al. 1982). Olfactometry studies showed that female Gulf Coast ticks migrate in significantly higher numbers toward a complement of CO_2 and volatile compounds from fed males compared to CO_2 or fed males alone (Kim 2004). These results support a hypothesis that questing female ticks are attracted to fed males and can discriminate between grazing cattle with fed males and those without. This hypothesis suggests that female attachment to cattle is not a random event but may be dependent on male distribution among hosts within the heard. In order to evaluate the male-female dynamics of individual adult tick attachment, an archival study was conducted using the Gulf Coast Tick infestations of untreated control cattle from eartag efficacy studies conducted in Texas from spring 1985 to fall 1991.

Materials and Methods

Data. To evaluate the dynamics of male-female attachment to pastured cattle, and test the hypothesis that female attachment is a non-random process tick infestation of untreated control cattle in a series of ear tag studies was examined. These data were collected in 1985, 1987, and 1991 from the Shay Ranch in Refugio County Texas. This ranch is situated in the Coastal Prairie region of Texas and the cattle used consisted of herefords and hereford-crosses. The cattle were individually restrained in a squeeze chut and inspected by hand, with special attention being placed on the head region. Consistatn data were collected on animal number, date, treatment, and the number of male/female Gulf Coast ticks present per animal for each study year.

In 1985, data were collected on nine separate occasions; May 20th, July 17th, August 1st, August 14th, August 29th, September 13th, September 27th, and October 10th. Figure 2-1 depicts the temporal distribution of the average number of male and female ticks per cow. Table 2-1 provides additional statistics for this same data.

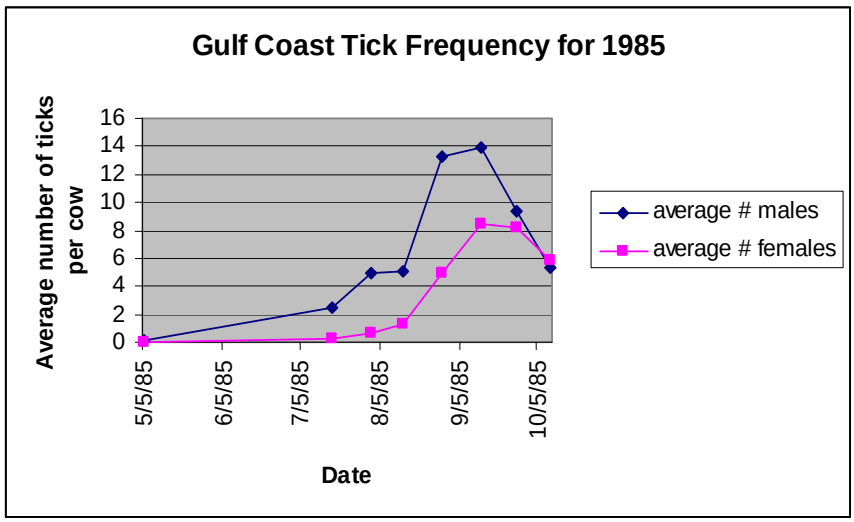

Figure 2-1. Average number of female and male ticks per cow by date for 1985.

Table 2-1. 1985 Gulf Coast tick infestation of untreated cattle on a coastal prairie ranch, Refugio Co. TX. (Pre-treatment counts were taken in May of the control group and in July of all cattle.)

date	total # cows	average # males	average # females	Min # males	Max # males	Min # females	Max # females
5-May	24	0.08	0.04	0	1	0	1
17-Jul	88	2.49	0.25	0	9	0	2
1-Aug	25	4.92	0.64	1	11	0	7
14-Aug	24	5.08	1.29	0	11	0	5
29-Aug	24	13.29	4.88	3	31	0	11
13-Sep	24	13.91	8.46	1	39	0	29
27-Sep	23	9.39	8.21	0	29	2	30
10-Oct	24	5.38	5.79	0	23	0	26

In 1987, data were collected on six separate occasions; June 19th, August 6th, August 20th, September 3rd, September 16th, and October 1st. Figure 2-2 depicts the temporal distribution of the average number of male and female ticks per cow. Table 2-2 provides additional statistics for this same data.

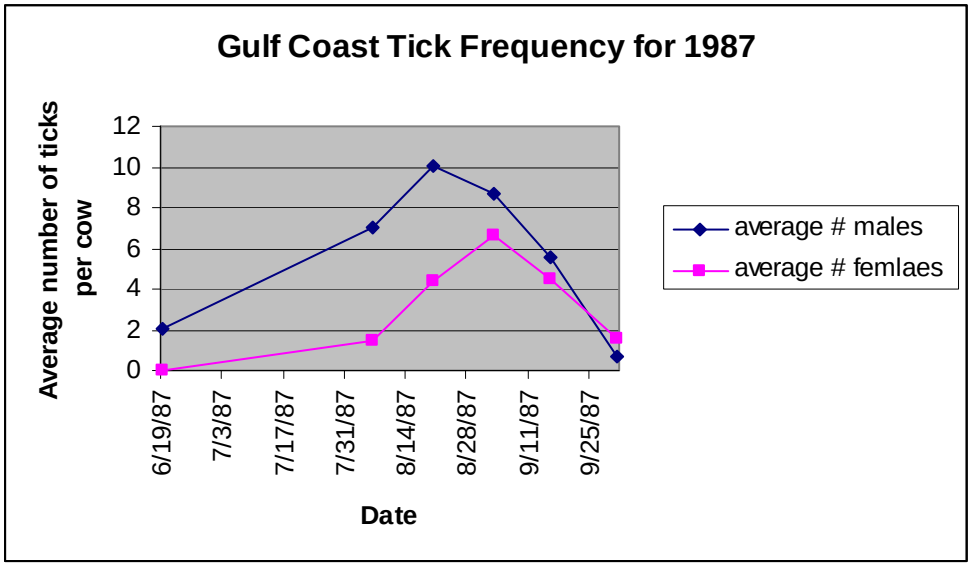

Figure 2-2. Average number of female and male ticks per cow by date for 1987.

Table 2-2. 1987 Gulf Coast tick infestation of untreated cattle on a coastal prairie ranch, Refugio Co. TX. (Pre-treatment counts were taken on June 19th of the control group and on Aug 6th of all cattle.)

Date	total # cows	average # males	average # females	Min # males	Max # males	Min # females	Max # females
19-Jun	25	2.04	0.04	0	11	0	1
6-Aug	74	7.04	1.46	0	38	0	8
20-Aug	25	10.08	4.4	1	27	0	11
3-Sep	25	8.68	6.64	0	29	1	15
16-Sep	25	5.52	4.51	0	36	0	13
1-Oct	25	0.68	1.56	0	6	0	7

In 1991, data were collected on six separate occasions; July 17th, August 1st, August 15th, August 28th, September 11th, and October 9th. Figure 2-3 depicts the temporal distribution of the average number of male and female ticks per cow. Table 2-3 provides additional statistics for this same data.

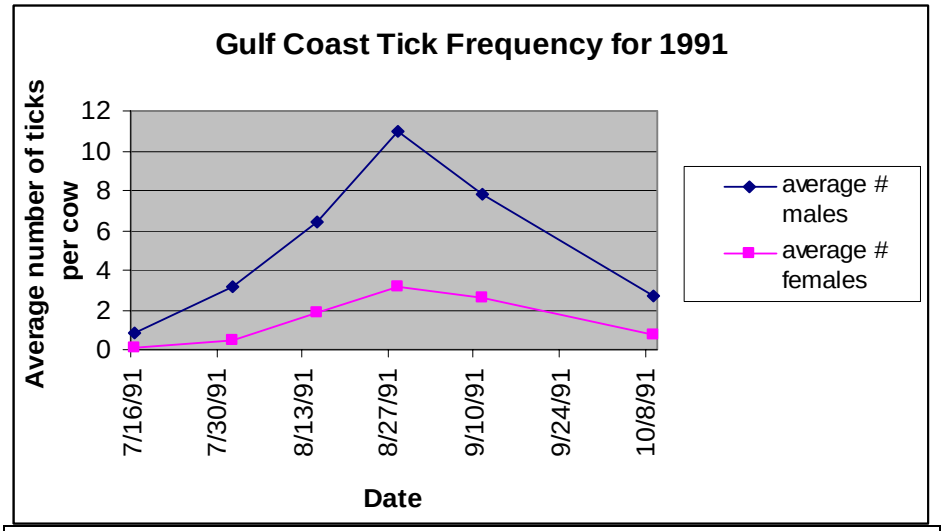

Figure 2-3. Average number of female and male ticks per cow by date for 1991.

Table 2-3. 1991 Gulf Coast tick infestation of untreated cattle on a coastal prairie ranch, Refugio Co. TX. (Pre-treatment counts were in July of all cattle.)

Date	total # cows	average # males	average # females	Min # males	Max # males	Min # females	Max # females
16-Jul	72	0.85	0.13	0	7	0	3
1-Aug	18	3.17	0.44	0	11	0	3
15-Aug	18	6.44	1.83	0	19	0	7
28-Aug	17	10.94	3.17	0	27	0	10
11-Sep	18	7.83	2.61	0	29	0	7
9-Oct	17	2.71	0.76	0	13	0	3

Once the data were summarized, they were arbitrarily divided into an early and late season based on the average number of male ticks attached per cow. It was noted that once the mean number of male ticks per cow was greater than or equal to ten, most cows were infested with ticks. Early season was therefore defined as the time period prior to the first date on which the average number of male ticks per cow was greater than or equal to ten. Late season was then defined as the time period after that point. For the data sets used this date occurred on August 29th 1985, August 20th 1987, and August 28th 1991.

Analysis. In order to test the hypothesis that female Gulf Coast ticks are attracted to hosts with feeding males, the data were analyzed in two ways; tick presence and average number of ticks per cow. In order to evaluate tick presence cattle were classified into four groups; those infested with male and female ticks, those infested with only male ticks, those infested with only female ticks, and those with no ticks (see Tables 2-4 & 2-5.)

Table 2-4. Relative frequency of cows with male and female ticks attached, only male ticks, only female ticks, and no ticks for early season 1985, 1987, and 1991. The summary column represents the relative frequency of these categories after pooling the three data sets.

	1985	1987	1991	Summary
Cows with male and female ticks	0.103	0.12	0.06	0.283
Cows with male ticks only	0.196	0.106	0.103	0.405
Cows with female ticks only	0.003	0	0.005	0.008
Cows with no ticks	0.136	0.043	0.125	0.304

Table 2-5. Relative frequency of cows with male and female ticks attached, only male ticks, only female ticks, and no ticks for late season 1985, 1987, and 1991. The summary column represents the relative frequency of these categories after pooling the three data sets.

	1985	1987	1991	Summary
Cows with male and female ticks	0.352	0.287	0.158	0.797
Cows with male ticks only	0.008	0.032	0.024	0.064
Cows with female ticks only	0.008	0.04	0.008	0.056
Cows with no ticks	0.016	0.045	0.02	0.081

The data for each year were initially analyzed separately. A Chi Squared test in the SPSS® 11.0 for Windows, SPSS Inc. was used to test the null hypothesis that the proportion of cattle in each group was equal. The null hypothesis was rejected at an alpha of less than 0.01 for all years and it was concluded that the proportions do differ. After determining that the data sets did not have a canceling effect on each other (the Simpson's paradox), the data were pooled and once again analyzed using a Chi Square test in SPSS® 11.0 for Windows. The null hypothesis was rejected and it was concluded that, like the year to year data, the proportions for the pooled data were also significantly different. A Chi Square test was also done to test the null hypothesis that proportions for early and late season data were equal. The null hypothesis was rejected at an alpha of less than 0.001.

After analyzing the data based on tick presence, the data were analyzed based on average number of male ticks per cow relative to female tick presence (see figure 2-4). The data did not vary greatly from year to year and were therefore pooled prior to analysis. To test the null hypothesis that the number of male ticks does not vary relative to the number of female ticks, a Fisher's protected least significant difference (LSD) procedure was conducted. This procedure consists of an analysis of variance followed by a Fisher's LSD procedure (Longnecker and Ott 2001). In the early season it was determined that all of the means were not equal and that they were all significantly different from one another. In the late season it was determined that all of the means were not equal, that the mean number of males with no females were significantly different from the mean number of males with greater than one female, and that the mean number of males with one female was significantly different from the mean number of males with

greater than one female. The mean number of males with no females and with one female was however not significantly different for late season data. This is probably due to the fact that by late season most of the cattle have a heavy infestation of both males and females resulting in most cows having more than one female tick.

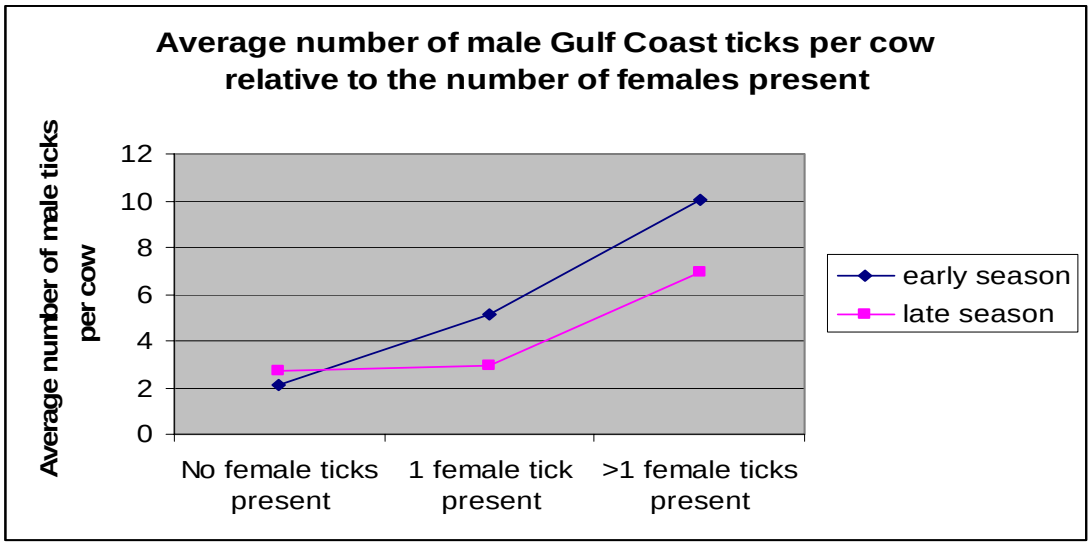

Figure 2-4. The average number of male Gulf Coast ticks per cow relative to the number of females on the cow.

Results

This study indicated that female Gulf Coast ticks in the field were more likely to be found on cattle infested with male ticks. In the early season only 0.8% of females were found on cattle in the absence of males, and only 3% in late season, indicating that females are attracted to cattle infested with male ticks (Table 2-6). It is also important to note that in the early season 41% of the cattle had only male ticks, whereas in the late season only 6% of the cattle had only male ticks, with 80% of the cattle having both male and female ticks present. This is likely due to the fact that female Gulf Coast ticks become active on the vegetation after males (Fleetwood 1985). A look at the average number of male ticks relative to female tick presence (Table 2-7) shows that female ticks are more likely to be found on cattle with more male ticks. This leads to the conclusion that questing female ticks are attracted to hosts with feeding males present.

Table 2-6. Relative frequency of cows in early and late season with male and female Gulf Coast ticks, male ticks only, female ticks only, and no ticks.

	Pooled data early	Pooled data late
Cows with male and female ticks	0.283	0.797
Cows with male ticks only	0.405	0.064
Cows with female ticks only	0.008	0.056
Cows with no ticks	0.304	0.081

Table 2-7. Average number of male Gulf Coast ticks per cow relative to the number of female ticks present.

	early season	late season
No female ticks present	2.11	2.75
1 female tick present	5.12	2.94
>1 female ticks present	10.04	6.92

CHAPTER III

STRAIN SPECIFIC PETRI DISH BIOASSAY OF FED MALE GULF COAST TICKS

Fed male Gulf Coast ticks of several geographic strains are believed to produce an AAP (Gladney et al.1974, Kim 2004). It has been suggested that the chemical profile of the volatile organic compounds produced by fed males believed to be AAP varies between different strains of Gulf Coast ticks (Kim 2004). It was also discovered that there is a genetic variation between the varying geographic strains of Gulf Coast ticks (Williams, 2002). This led to the hypothesis that female Gulf Coast ticks may not be equally attracted to fed males of different geographic strains. One technique that has been commonly used to determine female tick attraction is the Petri dish bioassay technique (Rechav et al. 1977, and 2000, Kim 2004). A Petri dish is divided into several sectors, treatments are placed into these sectors, ticks are released into the dish, and their location noted after a set time. Preliminary Petri dish studies were done and gradual trends were noted. Texas and Kansas females seemed to aggregate around Texas and Kansas males respectively two minutes post exposure. However, these trends were not statistically significant. Based on these general trends and the large replicate numbers used in prior Petri dish bioassay another Petri dish study was conducted with increased replication. The study was designed to examine whether female Gulf Coast ticks preferred males of the same geographic strain to those of other strains.

Materials and Methods

Ticks. Surplus adult *A. maculatum* reared in the Tick Research Laboratory, Department of Entomology, Texas A&M University under AUP number 2002-208 were used for part of this project. Two cows were clipped and six stockinets were glued onto each cow's top line using tag cement (Nasco Animal ID tag cement). Once the cement set, the cells were labeled (Figure 3-1). Seventy unfed adult males were put in each cell and allowed twenty four hours to attach, followed by eight days to feed prior to their use in the Petri dish bioassay. Ticks used for each experimental group were fed on the same cow. For example three cells on cow I were infested on day one with Texas, Oklahoma, and Kansas males to be used in the Texas female cross strain study, and on day two, the last three cells on cow I were infested with Texas, Oklahoma, and Kansas males to be used in the Oklahoma female cross strain study. The infestations were staggered so one female strain could be run per day starting on day nine.

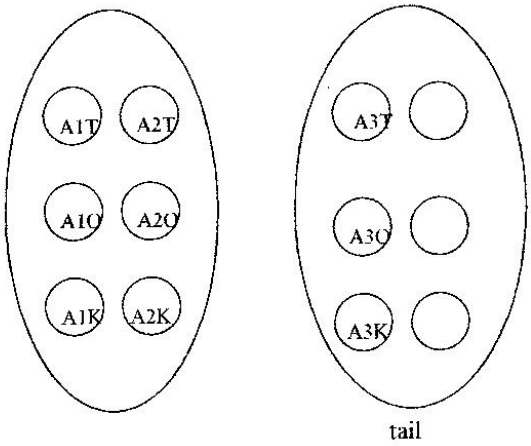

Figure 3-1. Cell arrangement on cows for experiment A. Cell A1T, infested on day one with 70 Texas males for use in experiment A1 (Texas female cross strain study); Cell A1O, infested on day one with 70 Oklahoma males for use in experiment A1; Cell A1K. Infested on day one with 70 Kansas males for use in experiment A1; Cell A2T, infested on day two with 80 Texas males for use in experiment A2 (Oklahoma female cross strain study); Cell A2O, infested on day two with 70 Oklahoma males for use in experiment A2; Cell A2K, infested on day two with 70 Kansas males for use in experiment A2; Cell A3T, infested on day three with 70 Texas males for use in experiment A3 (Kansas female cross strain study); Cell A3O, infested on day three with 70 Oklahoma males for use in experiment A3; Cell A3K, infested on day three with 70 Kansas males for use in experiment A3.

Bioassay. In order to determine if female Gulf Coast ticks prefer fed males of the same geographic strain, females were presented to males of three different geographic strains, Kansas, Oklahoma, and Texas, and the female's selection was recorded. Test arenas were Pyrex* brand (Corning Inc.) 150 x 20mm Petri dishes which were washed, rinsed with water, rinsed with acetone, and oven dried prior to each use. The test arenas were divided into 4 sectors on the bottom of the dish, so odor from the marker would not permeate the test arena. Eight feeding males from each strain were carefully removed from the appropriate cells off the skin of the cow and placed in three separate vials for transport. Five males of each strain were placed in separate small air-permeable chambers for bioassay; the remaining three were placed in three separate silanized glass vials and

used to verify that the fed males were producing the previously observed volatile organic compounds. Ticks in the silanized glass vials were exposed to a solid phase microextraction (SPME) filament for a minimum of two hours (Kim, 2004). When the next set of eight ticks was pulled three additional ticks were added to these vials. This process was continued until a total of 24 males were in each vial. The filaments were withdrawn two hours after the last three males were added. A clean vial was then placed on the end of the filament holder. Approximately twelve hours after collection each sample was injected into a HP 690Gas chromatography (GC) machine, with a BP-20 carbowax column (SGE, Ringwood, Australia)(Initial temperature at 50°C for 5 min, to 150°C rising 15°C/min, to 185°C rising 5°C/min, to 225°C rising 20°C/min; with 138KPa He gas carrier).Volatile compounds absorbed by the SPME were analyzed by gas chromatography and found to be comparable to those observed in previous studies (Kim 2004).

The test arenas were placed on a glass plate, covered with thin white parchment paper, to minimize reflection, heated with a heating pad and left for a minimum of four minutes (Figure 3-2). Test arena temperature was brought to approximately 90ºF (32.2ºC), to approximate the skin temperature of cattle observed in the lab. The temperature was monitored and recorded by an HOBO Pro Temp® (Onset Computer Corporation) temperature recorder.

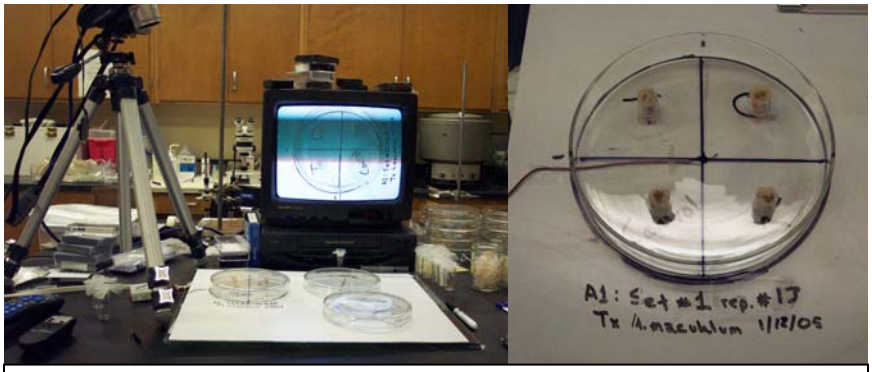

Figure 3-2. Petri dish bioassay lab set up.

As previously stated, the test arenas were divided into four sectors. At the onset of the experiment, sector one contained Texas strain fed males, sector two contained Oklahoma strain fed males, sector three contained Kansas strain fed males, and sector four contained a control vial with unfed strain specific (Texas) males. One Texas strain female was placed in the dish and her sector position relative to the chambers was recorded after two minutes. Unlike previously conducted Petri dish bioassays (Rechav et al. 1977, and 2000, Kim 2004) only one female was placed in the dish at a time to prevent any bias due to a possible follow-the-leader behavior. In order to provide additional reference all replicates were recorded with a camcorder. When the replicate was complete the males were moved to a new Petri dish and placement was rotated to the right by one sector. Since the amount of pheromone produced by fed males decreases approximately two to four hours after males are removed from the host, each group of removed fed males were used for thirteen replicates (an approximate time of one and a half hours) (Kim 2004). This was repeated, producing a total of 112 replicates for Texas

females, all completed within one day. A duplicate set of experiments were conducted for Oklahoma and Kansas females. A high mortality rate was observed in Kansas males resulting in only 6 sets or 86 replicates for the Kansas female replication.

Analysis. The treatments that were investigated included; Texas female exposure to fed Texas, Oklahoma, and Kansas males and unfed Texas males; Oklahoma female exposure to fed Texas, Oklahoma, and Kansas males and unfed Oklahoma males; and Kansas female exposure to fed Texas, Oklahoma, and Kansas males and unfed Kansas males. A Chi Square test in the SPSS* 11.0 for Windows (SPSS Inc.) was used to analyze the data and test the hypothesis that female Gulf Coast ticks do not respond equally to varying geographic strains of fed male Gulf Coast ticks. Data were also visually analyzed in the absence of replicates in which the female response was erroneous due to circling behavior in the test arena. One to three females per test population were observed to be circling the test arena and exclusion of these data did not alter the relative frequencies by more than 1 percent.

Results

Figure 3-3 females showed no preference to one geographic strain over another in these tests. They did however reconfirm female preference of fed males over unfed males. It is important to note that this preference was not statistically significant and that there is a high relative frequency of response to the unfed control group (Texas- 21%, Oklahoma- 16%, and Kansas- 9%.).

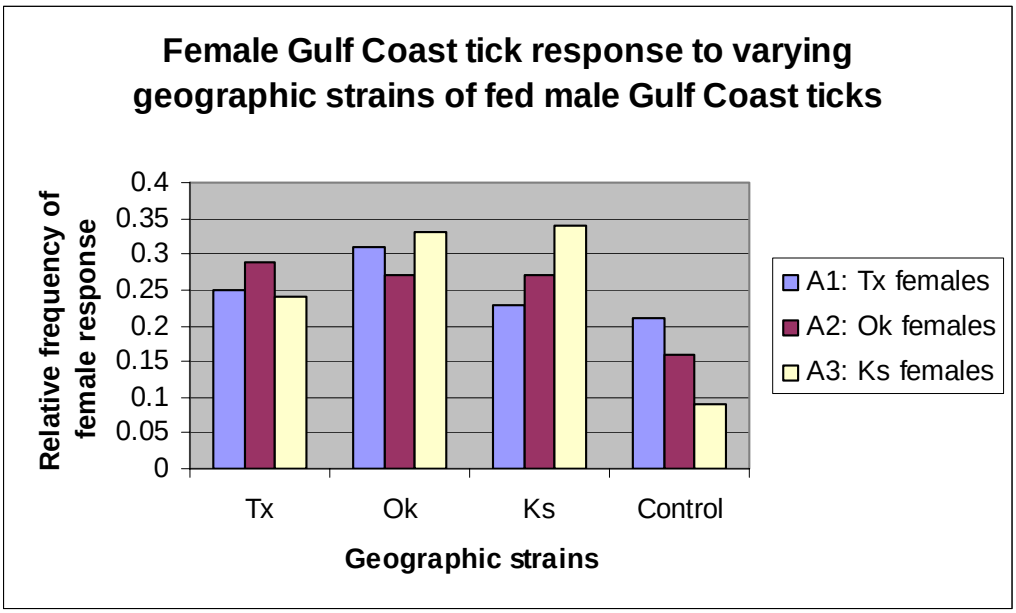

Figure 3-3. Relative frequency of female response to fed TX males, OK males, KS males, and control unfed males.

The data were analyzed separately for each female population tested. For the Texas and Oklahoma females, one was unable to reject the null hypothesis that female Gulf Coast ticks responded equally to all geographic strain at an alpha of 0.05 (Texas assumption significance = 0.426, Oklahoma assumption significance = 0.215). For the Kansas females, one was able to reject the null hypothesis at an alpha of less than 0.001. Based on the different outcomes of these analyses and the high relative frequency of response to the control treatment, one may conclude that the observed female response is not due primarily to the treatments.

These results suggest that the test females may not be able to distinguish differences between treatments in this system. This may be due to a bias in the experimental procedure. The Petri dish bioassay is conducted within a closed system which, in the case of Gulf Coast ticks, present two possible problems; a diffusion of potential volatile organic compounds unique to each male set as well as a lack of CO_2, carbon dioxide. In work done on other species of *Amblyomma* the AAP produced by the fed males contained highly volatile organic compounds, such as Benzaldehyde, Methal salicylate, and Phenylacetaldehyde (Price et al.1994, Norval et al.1992). In previously conducted Petri dish bioassays only one pheromone admitting treatment was presented to the females within the closed system (Rechav et al. 1977, and 2000, Kim 2004). The presence of three sets of males possibly emitting such volatile compounds may result in a rapid diffusion of these compounds throughout the Petri dish, making it difficult for the female tick to determine the origin of the pheromones. Also, work done by Kim (2004) indicated that Gulf Coast ticks respond better to AAP in the presence of CO_2 which is not present in significant amounts within the closed Petri dish. In order to test this hypothesis an open system will be needed.

CHAPTER IV

NON-SPECIFIC STRAINS PETRI DISH BIOASSAY OF FED MALE GULF COAST TICKS

The previous chapter discussed the response of female Gulf Coast ticks to several geographic strains of fed males, including their own. In this chapter we would like to explore the female response to several geographic strains in the absence of their geographically specific strain. This is important for two reasons; it may help us find the ideal strain of ticks to use as a model for pheromone replication to be used in pheromone enhanced Gulf Coast tick control, and it will identify if added control methods are needed for possible introduced tick strains.

In order to examine the behavior of Gulf Coast tick females in the absence of a male of the same geographic strain a second Petri dish experiment was conducted. Preliminary experiments were also done for this experiment, although these tests were not statistically significant possible trends were observed. The females tended to prefer the fed males over the unfed males and the empty chamber, alluding to the possibility that in the absence of their geographically specific male, that females will respond to either strain of fed males. Based on these general trends and the large replicate numbers used in prior Petri dish bioassay, another Petri dish study was conducted with increased replication. The experiment was run in the same manor as the previous chapter's experiment, however, the males of the same geographic strain as the females were removed.

Materials and Methods

Ticks. Surplus adult *A. maculatum* reared in the Tick Research Laboratory, Department of Entomology, Texas A&M University under AUP number 2002-208 were used for this part of this project. One cow was clipped and six stockinets were glued onto its top line using tag cement (Nasco Animal ID tag cement). Once the cement set the cells were labeled (Figure 4-1). Seventy unfed adult males were put in each cell and allowed twenty four hours to attach, followed by eight days to feed prior to their use in the Petri dish bioassay. Ticks used for each experimental group were fed on the same cow for the same period of time. For example, two cells on cow III were infested on day one with Oklahoma and Kansas males to be used in the Texas female non-specific strain study (B1), on day two two more cells on cow III were infested with Texas and Kansas males to be used in the Kansas female non-specific strain study (B2), and on day three the last two cells on cow III were infested with Texas and Oklahoma males. The infestations were staggered so one female strain could be run per day starting on day 9.

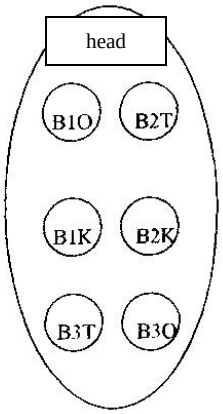

Figure 4-1. Cell arrangement on cows for experiment B. Cell B1O, infested day seven with 70 Oklahoma males for use in experiment B1 (Texas female non-specific strain study); Cell B1K, infested day seven with 70 Kansas males for use in experiment B1; Cell B2T, infested on day eight with 70 Texas males for use in experiment B2 (Oklahoma female non-specific strain study); Cell B2K, infested on day eight with 70 Kansas males to be used in experiment B2; Cell B3T, infested on day nine with 70 Texas males to be used in experiment B3 (Kansas female non-specific strain study); Cell B3O, infested on day nine with 70 Oklahoma males for use in experiment B3.

Bioassay. In order to determine if female Gulf Coast ticks prefer fed males of one geographic strain over another, females were presented to non-specific fed males and the female's selection was recorded. Test arenas were Pyrex* brand (Corning Inc.) 150 x 20mm Petri dishes that were washed, rinsed with water, rinsed with acetone, and oven dried prior to used. The test arenas were divided into 4 sectors on the bottom of the dish, so odor from the marker would not permeate the test arena. Eight feeding males from each strain were carefully removed from the appropriate cells off the skin of the cow and placed in three separate vials for transport. Five males of each strain were placed in separate small air-permeable chambers for bioassay, the remaining three were placed in three separate silanized glass vials, to verify that the fed males were producing the previously observed volatile organic compounds. Ticks in the silanized glass vials were

exposed to a solid phase microextraction (SPME) filament for a minimum of two hours (Kim 2004). When the next set of eight ticks was pulled three additional ticks were added to these vials. This process was continued until a total of 24 males were in each vial. The filaments were withdrawn two hours after the last three males were added. A clean vial was then placed on the end of the filament holder. Approximately twelve hours after collection each sample was injected into a HP 690Gas chromatography (GC) machine, with a BP-20 carbowax column (SGE, Ringwood, Australia)(Initial temperature at 50°C for 5 min, to 150°C rising 15°C/min, to 185°C rising 5°C/min, to 225°C rising 20°C/min; with 138KPa He gas carrier).

The test arenas were placed on a glass plate, covered with a thin white parchment paper (to minimize reflection from the glass plate) heated with a heating pad and left for a minimum of four minutes. Test arena temperature was brought to approximately 90ºF (32.2ºC), the approximate skin temperature of cattle. The temperature was monitored and recorded by an HOBO Pro Temp® (Onset Computer Corporation) temperature recorder. In each Petri dish sector one contained the Oklahoma strain fed males, sector two contained the Kansas strain fed males, sector three contained unfed Texas males, and in sector four contained an empty control vial. One female was placed in the dish and her sector location with respect to the treatment was recorded after two minutes. In order to provide additional reference all replicates were recorded with a camcorder. When the replicate was complete the males were moved to a new Petri dish and placement was rotated to the right by one sector. Since the amount of pheromone produced decreases approximately two to four hours after males are removed from the host, each group of removed fed males were used in only thirteen replicates (an approximate time of a hour

and a half) (Kim 2004). This was repeated producing a total of 112 replicates for Texas females, all completed within one day. A duplicate set of experiments was conducted for Oklahoma and Kansas females. A high mortality rate was observed for Kansas males resulting in only 7 sets or 98 replicates for the Texas female replicates and 6 sets or 84 replicates for the Oklahoma female replication.

Data Analysis. The treatments that were investigated included; Texas female exposure to Oklahoma and Kansas fed males, unfed Texas males, and a blank chamber; Oklahoma female exposure to fed Texas and Kansas males, unfed Oklahoma males, and a blank chamber; and Kansas female exposure to fed Texas and Oklahoma males, unfed Kansas males, and a blank chamber. A Chi Square test in the SPSS* 11.0 for Windows (SPSS Inc.) was used to analyze the data and test the hypothesis that female Gulf Coast ticks do not respond equally to varying geographic strains of fed male Gulf Coast ticks .

Results

As seen in table (Table 4-1) females tended to prefer fed males over the unfed males and the empty chambers. In general females responded the least to control 2, the empty chamber. It is, however, important to note that the mean responses were not significantly different.

Table 4-1. The relative frequency of female response to fed non-geographically specific males. The black boxes represent fed males that were not exposed to the females.

Experiment	TX	OK	KS	Control 1	Control 2
B1: TX females		0.245	0.337	0.224	0.194
B2: OK females	0.417		0.262	0.143	0.179
B3: KS females	0.264	0.3		0.255	0.182

The data were analyzed separately for each population of females tested. For Texas and Kansas females, one was unable to reject the null hypothesis that female Gulf Coast ticks responded equally to all geographic strains presented at an alpha of 0.05 (Texas assumption significance = 0.217, Kansas assumption significance = 0.357). For Oklahoma females, one was able to reject the null hypothesis at an alpha of 0.002. Based on the different outcomes of these analyses and the high relative frequency of response to both controls one may conclude that the observed female response is not due primarily to the treatments.

As stated in the previous chapter, these results suggest that the test females may not be able to distinguish differences between treatments in this test system. This may be due to a bias in the experimental procedure. The Petri dish bioassay is conducted within a closed system which, in the case of Gulf Coast ticks, presents two possible problems; a build up of volatile organic compounds as well as a lack of CO_2.

CHAPTER V

SUMMARY AND DISCUSSION

Archival Study

Gulf Coast ticks have developed a unique survival and procreation strategy. These studies show that female Gulf Coast ticks are more attracted to cattle with feeding males than to without males. Further this preference is supported by y-tube olfactometry studies showing synergistic attraction of fed males and CO_2 than either stimulus alone (Kim 2004). A female's ability to detect hosts with males present allows them to feed and mate on host in a fairly limited period of time. This is important to a Gulf Coast tick's survival because females become very large after feeding and are therefore prone to being groomed off by the host.

Understanding male-female host interactions can help aid in controlling Gulf Coast tick populations. If the AAP which is being produced by the fed males could be isolated, it could be incorporated into pesticide impregnated tags and collars. The AAP enhanced tags could greatly increase the efficacy of the tags currently being used by attracting ticks close to the pesticide. The AAP would also greatly increase the efficacy of off-host control methods such as CO_2 traps.

Further research in this subject is needed primarily in two areas; pheromone isolation and immature attachment patterns in the field relative to adult fed males. Since the volatile organic compounds produced by Gulf Coast ticks have been analyzed by a GC it may be possible to identify the compound by comparing it to known components of

pheromones produced by other *Amblyomma* species. Once identified the compound could tested using methods described by Kim (2004).

In order to determine if AAP may be used to aid in control of immature populations, their host preference in the field must be better understood. According to Wexler, when placed on a bovine host immatures have been observed to aggregate near fed males (Departmental Seminar Texas A&M, 2004). Immature attraction to host relative to adult male presence on pastured cattle has not, however, been studied. Analyzing immature attachment patterns in the field may prove to be more challenging, however, since larval and nymph stages are often difficult to locate on cattle.

Petri Dish Bioassays

Petri dish studies are not appropriate to test female preference over several groups of pheromone producing males. It is, however, necessary to determine female response to varying geographic strains in order to determine which strain is best to use as a model for AAP synthesis for use in population control and monitoring.

In order to test the hypothesis that female Gulf coast ticks are not equally attracted to fed males of different geographic strains an open system able to incorporate a controlled CO_2 stimuli must be used. A multi-treatment olfactometer is needed that can incorporate the Peti dish design with an Y-tube olfactometer (figure 5-1) (Soneshine et al. 1982, Kim 2004). This design would allow for the addition of a regulated CO_2 stimuli as well as preventing the diffusion of volatile compounds. Females would be released into a central chamber and presented with several pathways (tubes attached to treatment chambers) to choose from.

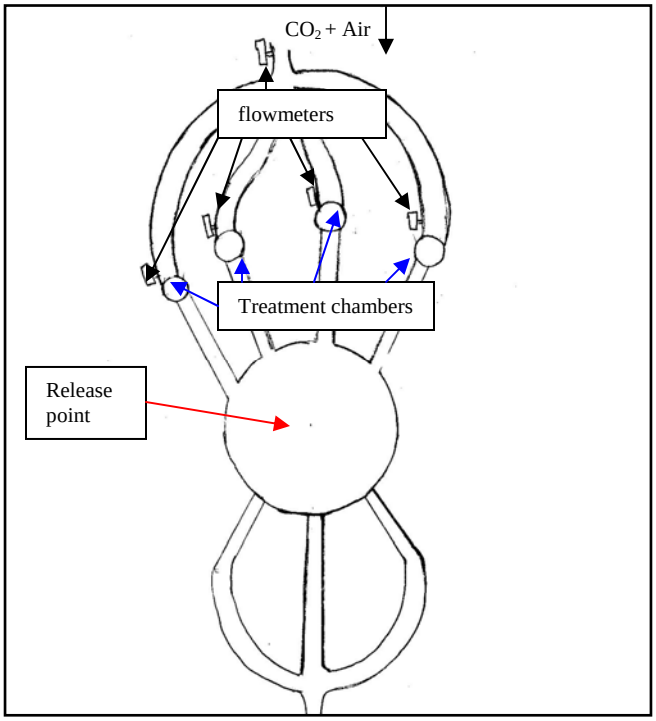

Figure 5-1. Multi-treatment olfactometer. CO_2 plus air is pulled and/or pushed thought the system adding stimuli and removing slowly diffusing chemicals produced by the treatment.

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APPENDIX I

Frequency data for Archival study (Chapter II).

Early season control ear tag frequencies

Early season			
	1985	1987	1991
Cows with male and female ticks	38	44	22
Cows with male ticks only	72	39	38
Cows with female ticks only	1	0	2
Cows with no ticks	50	16	46

Late season control ear tag frequencies

Late Season			
	1985	1987	1991
Cows with male and female ticks	87	71	39
Cows with male ticks only	2	8	6
Cows with female ticks only	2	10	2
Cows with no ticks	4	11	5

Average number of male Gulf Coast ticks present relative to female ticks early season , 1985, 1987, 1991.

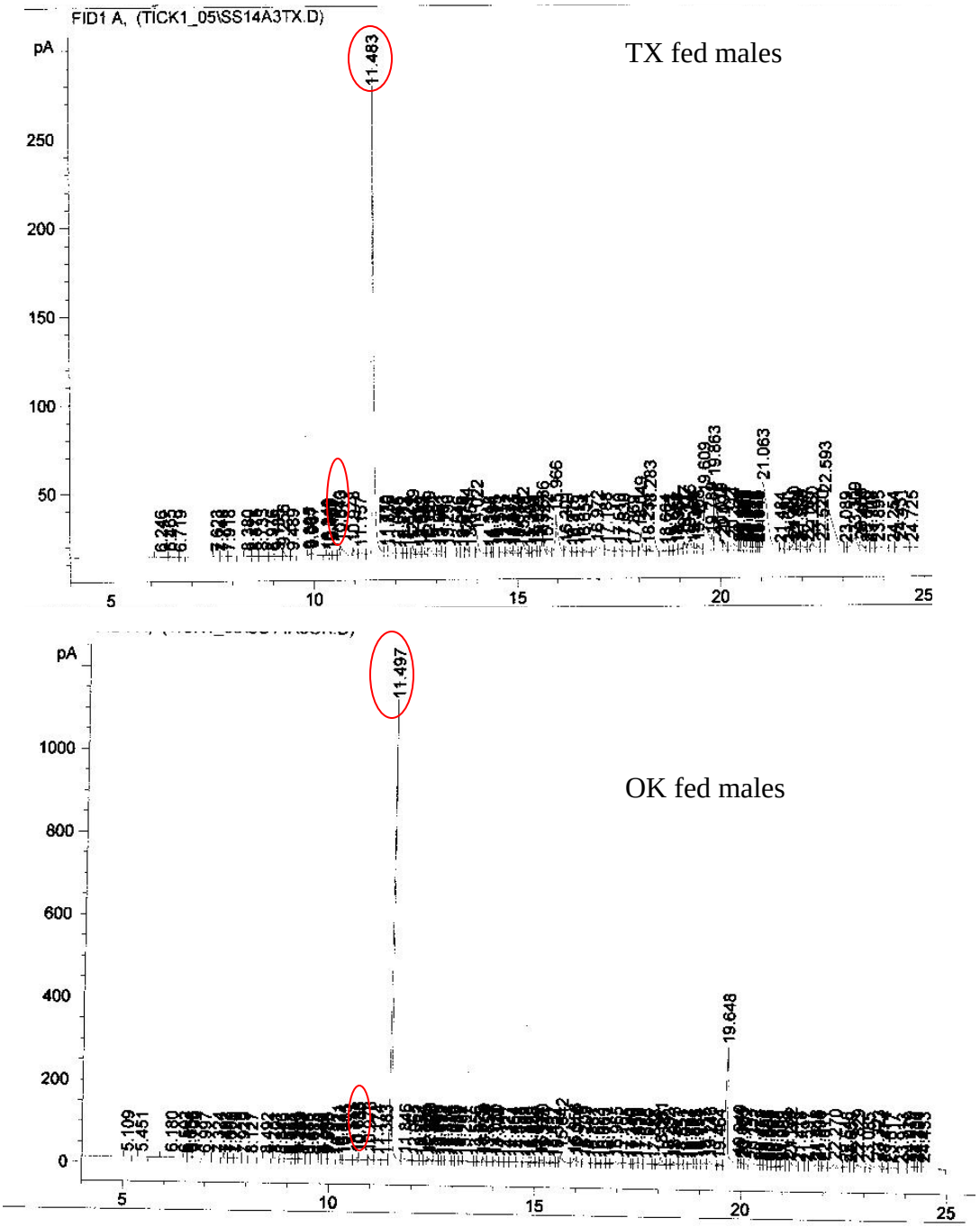
Average number of males present per cow early season			
	1985	1987	1991
No female ticks present	2.18	3.17	1.07
1 female tick present	4.31	5.2	5.15
>1 female ticks present	6.19	10.77	6.73

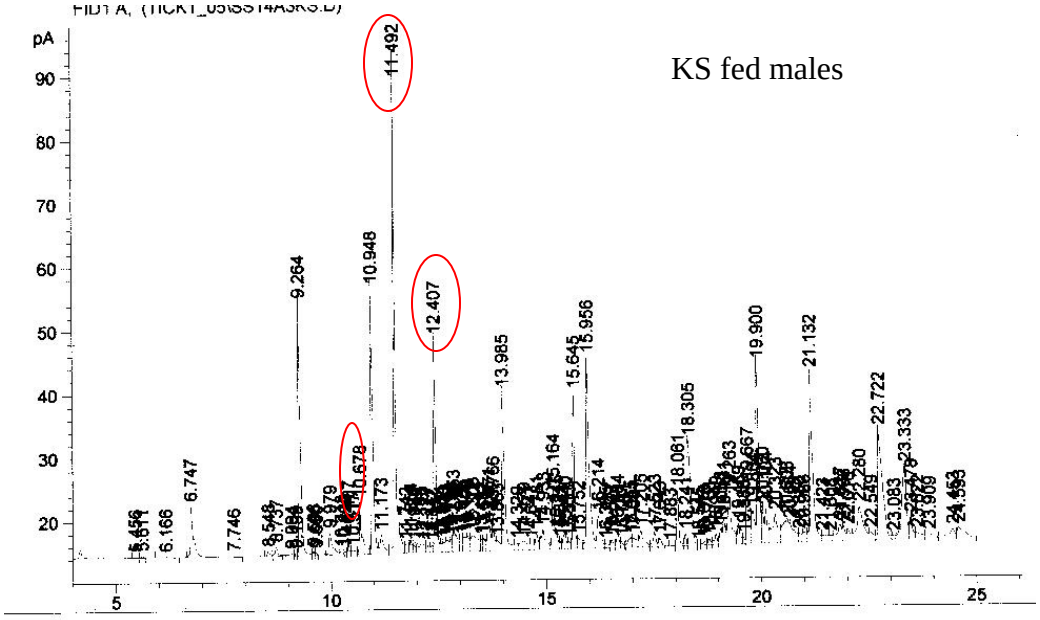
Average number of male Gulf Coast ticks present relative to female ticks late season 1985, 1987, 1991.

Average number of males present per cow late season			
	1985	1987	1991
No female ticks present	2	1.5	3.27
1 female tick present	3.72	3.2	7.88
>1 female ticks present	11.86	8.87	8.44

APPENDIX II

Gas Chromotography output of solid phase microextraction head collection sample of 8 day fed Gulf Coast ticks (Chapter III & IV).

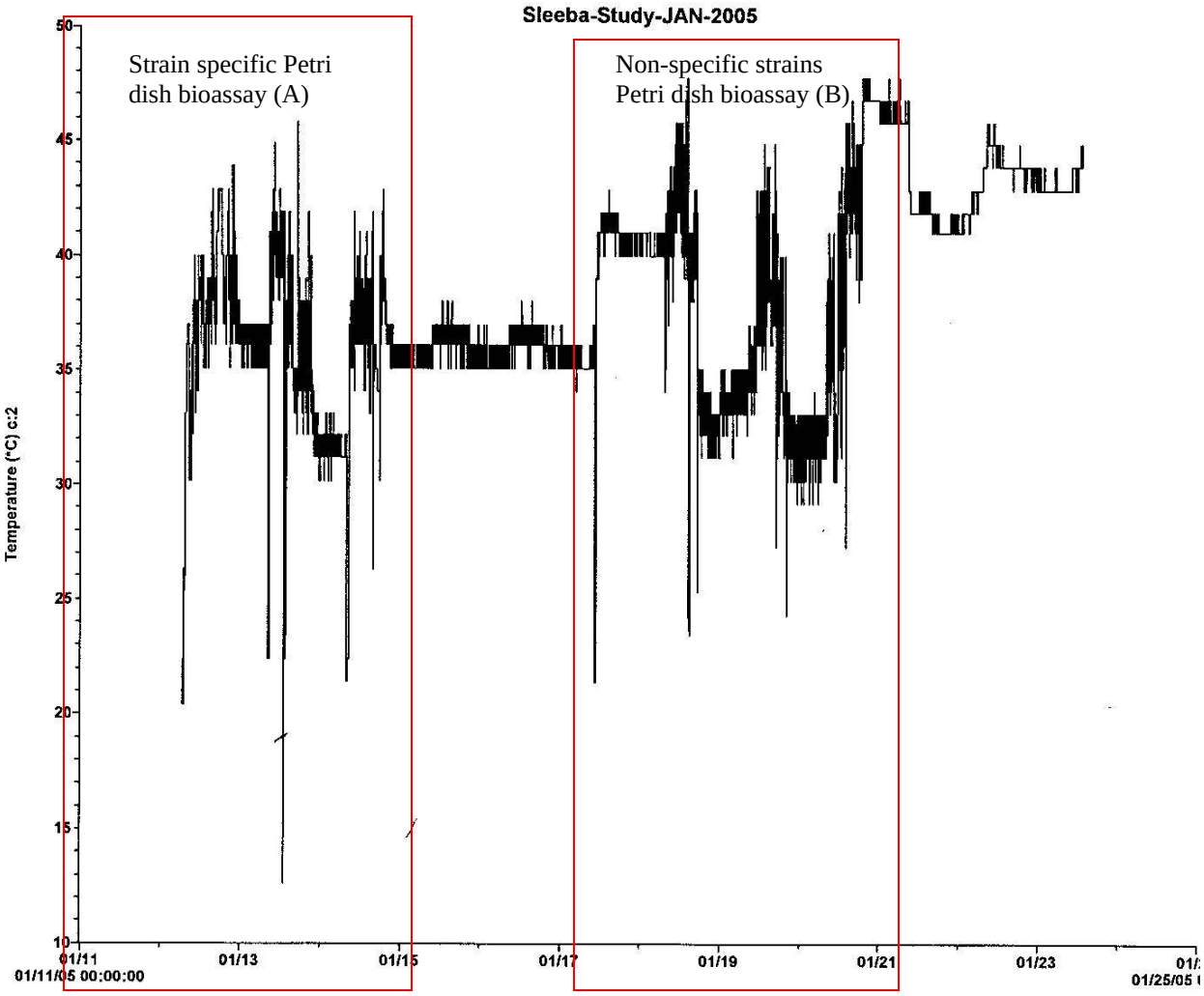




All GC runs were done on a HP 6980 equipped with a BP-20 carbowax column. Circled peaks indicate previously identified peaks of interest produced by fed male Gulf Coast ticks (Kim, 2004).

APPENDIX III

HOBO Pro Temp® temperature recordings for strain specific and non-specific Petri dish bioassay (Chapter III & IV).



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