THE ETHOLOGICAL BASIS OF SOUND AS
A HOST DETECTION CUE
IN IXODID TICKS

By

WILLIAM DONALD BUNN

Bachelor of Science in Agriculture

New Mexico State University

Las Cruces, New Mexico

1979

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
July, 1983

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Thesis Approved:

R. W. Barker
Thesis Adviser

John R. Sauer

J. H. Young

Herb Pohl

Norman N. Durham
Dean of Graduate College

ACKNOWLEDGMENTS

I would like to express my gratitude to my adviser, Dr. R. W. Barker, for his insight that created this study and his patience while I developed and conducted the experiments. Dr. Barker's understanding of ticks and his open mind to new ideas created an atmosphere of learning and growth.

For their expert counsel and guidance, I thank my committee members, Dr. J. R. Sauer, Dr. J. H. Young and Dr. H. A. Pohl. Finally, Thomas Underwood and David Strebe provided crucial technical assistance that is greatly appreciated.

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CHAPTER I

INTRODUCTION

The mechanisms by which ticks perceive their environment and are able to respond to stimuli in order to seek a host, have not been fully established. Although it is generally accepted that olfaction is the dominant factor in the host-seeking behavior of ticks (Waladde & Rice, 1982), clearly there are many other sensory cues that may be significant. A sensory stimulus can be classified as being abiotic or biotic in origin. It is the latter with which ticks find a host. To date, responses to light (Lees, 1948; Wilkinson, 1953; George, 1963), heat (Lees, 1948), and objects in motion (Lees, 1948), have been documented. The role of sound in host detection has been studied in argasid ticks (Webb et al. 1977). However, the importance of sound stimuli in ixodid tick host detection has not received critical attention. Therefore, the objective of this investigation was to determine the ability of ixodid ticks to perceive and utilize sound as a single sensory stimulus.

CHAPTER II

METHODS

The sound equipment and monitoring devices were similar to those used by Webb et al. (1977). The experiments were conducted within an anechoic chamber. Dimensions of the chamber are 2.44 x 1.22 x 1.83m high and is of wood frame construction. Exterior walls of the chamber were constructed of 1.27cm gypsum drywall (sides, ends and outside), galvanized screen wire, 0.1mm thick sheet plastic, 2.53cm thick polure-thane foam (insede lining and in-wall insulation) and 1.27cm plywood (top and bottom).

A stage was constructed on which individual ticks were placed to observe their response to sound stimuli. The stage consisted of 0.64cm thick circular glass 30.48cm in diameter. A Radio Shack® Model Solo-5 stereo loud speaker with piezo tweeter was positioned 40.64cm from the center of the stage. The speaker was covered with a 2.54cm thick polyurethane foam pad with a 2.54cm vertical slit to concentrate the sound signal. the stage rested within an open-ended, framed, cubic structure 45.72cm on each side. The cube was made of 5.08 x 10.16cm wood. The speaker was placed in the open end with 2.54cm polyurethane foam in two of the four vertical sides. Speaker placement represented zero degrees.

The entire stage rested upon a wooden table 1.22 x 0.61 x 0.91m high with butyl rubber pads 0.32cm thick placed at the bottom and top of each leg and a 5.08cm thick styrofoam pad was placed under the stage to minimize vibrations. Access to the inside of the chamber was made possible by using Jelco® OB sleeves that were externally mounted at 15.24cm diam. holes under a 30.48 x 15.40cm glass observation window.

Illumination during experimentation was 0.38 µeinsteins/second. Temperature and relative humidity in the chamber were maintained at $23 \pm 1.5^{\circ}C$ and 70 ± 4 percent, respectively. The sound intensity was 70 ± 2dB at the center of the stage.

Nymphs and adults of three tick species were studied; *Dermacentor variabilis*, Say (American dog tick), *Amblyomma americanum*, (L.) (lone star tick), and *Amblyomma maculatum*, Koch (Gulf Coast tick). The ticks were fed on rabbits as larvae and calves as nymphs and maintained in the laboratory at 84 ± 4 percent relative humidity and $24 \pm 2^{\circ}C$ with a photoperiod of 14L:10D.

At the start of each trial, ticks were placed individually at the center of the stage, the sound system turned on, and the point at which the ticks left the stage was noted in degrees from zero. The time limit for each trial was 15 minutes for nymphs and 10 minutes for adults. Control experiments were done with the speaker turned on, but without sound signal output.

Host/Habitat Recordings Trials

Tape recordings of the sounds made by humans walking through leaf-litter were made using a Radio Shack® CRT-80A portable cassette tape player and Sony® LNX Type I cassette tapes. These recordings were played over a Radio Shack® Model SA-10 solid state stereo amplifier. The output was displayed visually using a Tektronix® Model 5113 dual beam storage oscilloscope after being amplified by a Grass® Model P15 differential AC preamplifier.

Repetitions were performed for groups of nymphal ticks ages 1-5 weeks post molt (WPM). Adult tick trials were carried out for ages 1, 2, 3, 6, 8, 10, 12 and 14 WPM. Trials were also completed for groups of adult D. variabilis without tarsi I, without pretarsi I, and lowered sound intensity (60 ± 2dB center stage). Tarsi and pretarsi were removed using a dissecting microscope and heated surgical blade. A trial for adult D. variabilis during which the speaker was placed at the point corresponding to 180 degrees was carried out. Amblyomma spp. aged 3 WPM were desiccated for 1-3 hours in a Nalgene® desicator containing calcium sulfate, before testing to determine if responses could be induced.

Tick Response to Individual Frequencies

The host/habitat recordings were analyzed visually using an oscilloscope. The behavioral response of D. variabilis ticks to fifteen individual frequencies (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 5.0, 10.0, 15.0, and 22.0 KHz), generated by a Hewlett-Packard® Model 200AB audio oscillator was examined. The lower frequencies (<5.0 KHz) were equalized by a Radio Shack® 5 band graphic equalizer to ensure a flat response over the entire frequency range.

Tick Response to Substrate-Borne Vibrations

Trials observing the behavioral response of adult D. variabilis to vibrations introduced into the stage at zero degrees were performed. Frequencies of 0.1, 1.0, 10.0, and 22.0 KHz were generated by a Goodman® drive, sine wave vibration generator. This electrodynamic vibrator acts to transduce the output of the oscillator into vibrational frequencies within the glass stage (Hueter & Bolt, 1960). The vibrations were generated in the direction from zero to 180 degrees at an intensity of 70 ± 1 dB center stage. Testing and analysis was accomplished in the same manner as for the trials concerning air-borne sounds.

Statistical Analysis

Statistical analyses were carried out using Rayleigh's test and the V test as described by Zar (1974). The trials were deemed significant if the calculated values corresponded to a probability level of 5 percent or less compared to controls. The significance for the V test's critical value V, is expressed as the probability that the experimental mean vector angle is different than the expected mean vector angle (zero degrees is the expected angle in all tests except the reverse angle trial where the origin equals 180 degrees). The significance for Rayleigh's critical value r is expressed as the probability that the sample has a unimodal directional response.

CHAPTER III

RESULTS

Host/Habitat Recordings

When nymphal ticks of each species were subjected to recorded sounds, no positive mean directional response was observed. This was in contrast to observed behavioral responses of adults subjected to stress and nonstress situations.

In all age groups, female *D. variabilis* were generally more responsive than males (Table 1). Although Rayleigh's test failed to show any mean directional response for male *D. variabilis*, the V test did reveal that the mean angles were significantly close to zero for all but the first and last weeks of testing.

Male *A. maculatum* responses were not significant compared to controls with either analysis, however, the females showed a peak response period between 8 and 10 WPM (Table 2).

The response of *A. americanum* individuals paralleled that of *A. maculatum* with two notable exceptions (Table 3). There was a significant directional response at 12 WPM for males, even though the V test casts some doubt on this datum. Secondly, the peak response for female *A. americanum* was during 10, 12, and 14 WPM periods (Table 4).

The removal of tarsi I in adult D. variabilis inhibited their behavioral response to the recordings compared to the two control groups (Table 5). One group had tarsi IV removed, another group was comprised of intact ticks. Furthermore, groups with one tarsus I removed lost the ability to orient to the sound stimulus.

The removal of pretarsi I in adult D. variabilis also inhibited their response, but to a much lesser extent than with the tarsi (Table 6). Control groups were similar to those in the tarsus removal study.

Lowering the sound intensity at the center of the stage from 70 $\pm$ 2dB to 60 $\pm$ 2dB reduced tick behavioral responses slightly but significantly (Table 7). This intensity reduction is equivalent to increasing the distance between the speaker and center stage from 40.64cm to 165.16cm.

Speaker placement at a point corresponding to 180 degrees from the origin resulted in minor changes in the behavioral response of D. variabilis ticks (Table 8). Male ticks failed to positively respond in either speaker location.

Desiccation of Amblyomma spp. at a relative humidity of <5 percent, showed that a response could be induced, but only after 3 hours (Table 9). Using the V test, only A. americanum ticks demonstrated valid responses.

Tick Response to Individual Frequencies

Analysis of host/habitat recordings revealed a total of 21 frequencies, 75 percent of which are the five listed in Table 10.

Male and female D. variabilis responded to frequencies at and below 1.0 KHz (Table 11). As in the host/habitat recordings trials, the female response was stronger than that of the males. A positive response was obtained with females at 2.0 KHz. There were no significant responses in males at frequencies above 1.0 KHz.

Tick Response to Substrate-Borne Vibrations

No trial resulted in a significant directional response in either the V test or Rayleigh's test. When 180 degrees was used for the expected mean vector angle in the V test, probability of 0.026 was obtained for 22.0 KHz. No significance was shown using Rayleigh's test for this frequency.

CHAPTER IV

DISCUSSION

The sensation of sound impinging upon an insect's body can be perceived by two broad "hearing" organ types (Haskell 1961). There may be organs comprised of groups of chordotonal sensilla, such as tympanal organs and Johnston's organ. Other more widely scattered structures are mechanoreceptive hair sensilla of several morphologic types. The literature to date, has provided no evidence to indicate the presence of chordotonal organs in ticks. Conversely, the sensory function of setal mechanoreceptors have been shown to be tactile and proprioreceptive, but the more complex process of sound reception and discrimination by sensilla in Acarina has yet to be demonstrated.

The data of this study ethologically shows, D. variabilis has the ability to sense and orient to certain sound frequencies (Tables 1 and 4), The physiological basis for such responses appears to be related to structures located on tarsi I (Tables 5 and 6). Whether Haller's organ is the actual otic organ remains to be examined, but its involvement seems reasonable due to anatomical differences in setal types, number and arrangement between species (Bruce, 1971; Waladde & Rice, 1982; Barker et al., unpublished). The requirement for both tarsi I to be present for orientation to sound to occur (Table 5) indicates sound is perceived as a scalar quantity; each sensory organ receives stimuli with no directional component.

Differences in male and female D. variabilis response were dramatic and widespread (Tables 1, 4 - 8 and 11). It should be noted that there are fewer physical differences in tarsi I between sexes on which to base the observed phenomena than between species (Barker et al., unpublished).

The fact that behavioral responses of ticks to sound varies with age could be due to physiological changes associated with reproduction and/or nutritional status. The former would logically be the greatest factor since no response was seen in nymphs. Furthermore, a peak response in adult ticks over time, was observed (Tables 1- 4). The maximal and minimal response periods coincide with those found for olfaction (Holscher et al., 1980). If a tick's nutritional state is the controlling factor, it would seem likely that older ticks within the age range of this study would have stronger responses.

Lower sound stimulus intensity resulted in decreased directional orientation (Table 7). This effect points towards a distance component of sound stimuli. Ticks could use sound to orient to a host only at close range. Other stimuli such as olfaction would dominate responses at distances. As an alternative explanation, sensilla embedded within the integument would respond only when a stimulus of sufficient intensity is present. This response, dependent upon the integument acting as a diffuse tympanal membrane, would have a relatively high threshold of activation. No anantomical evidence for this has yet been found on tarsi I of ticks, but Haskell (1961) states that sensilla of this type are found in all orders of insects and have an effective frequency range of 0.05 to 1.0 KHz.

No substrate-borne vibrations of frequencies tested induced a positive direction response in _D. variabilis_. It was noted that a frequency of 22.0 KHz had a repulsive effect on the ticks. During experimentation, the ticks, once placed upon the stage, behaved differently from those in the previous sound studies in two characteristic ways. Typically, in the air-borne sound studies, a one to three minute period of akinesis was seen immediately following contact with the stage and secondly, there was a characteristic questing attitude (waving the first pair of legs). In the vibration study, this akinetic period was almost nonexistent and questing was virtually absent in response to substrate-borne stimuli. These observations suggest a proprioceptive response rather than reception and discrimination of sound stimuli.

REFERENCES

Bruce, W. A. (1971). Posterior capsule of Haller's organ in the lone star tick, Amblyomma americanum (Acari:Ixodidae). Fla. Entomol. 54, 65-72.

George, J. E. (1963). Responses of Haemaphysalis leporis-palustris to light. Adv. Acarol. 1, 425,430.

Haskell, P.T. (1961). Insect Sounds. pp. 62-102. Chicago: Quandrangle.

Holscher, K. H., Gearhart, H. L. & Barker, R. W. (1980). Electrophysiological responses of three tick species to carbon dioxide in the laboratory and field. Ann. Entomol. Soc. Amer. 73, 288-292.

Hueter, T. F. & Bolt, R. H. (1960). Sonics. pp. 279-280. New York, London: John Wiley & Sons.

Lees, A. D. (1948). The sensory physiology of the sheep tick, Ixodes ricinus, L. J. Exp. Biol. 25, 149-207.

Waladde, S. M. & Rice, M. J. (1982). The sensory basis of tick feeding behavior. In Physiology of Ticks, (ed. F. Obenchain & R. Galun) pp. 71-118. New York: Pergamon Press.

Webb, J. P., Jr., George, J. E. & Cook, B. (1977). Sound as a host-detection cue for the soft tick Ornithodorus concanensis. Nature. 265, 443-444.

Wilkinson, P. R. (1953). Observations on the sensory physiology and behavior of larvae of the cattle tick, Boophilus microplus (Can.) (Ixodidae). Aust. J. Zool. 1, 345-356.

Zar, J. H. (1974). Biostatistical Analysis. pp. 310-328, 567-573. Englewood Cliffs, N. J.: Prentice-Hall.

APPENDIXES

Table 1

Comparison of the positive directional response of adult Dermacentor variabilis to sound stimuli[1/] [2/] [3/]

Sex	X No. tested	Weeks Post Molt 1	2	3	6	8	10	12	14
Female	12	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	0.0022	
Male	14	N.S.	0.041	0.037	0.23	0.18	0.023	N.S.	

1/ Recorded sound of human movement in leaf litter.

2/ V test from J.H. Zar (1974).

3/ Control response was based upon tick behavior in the absence of sound stimuli. Control probability was not significant (N.S.).

Table 2

Comparison of the positive directional response of adult *Amblyomma maculatum* to sound stimuli[1/] [2/] [3/]

Sex	No. X tested	Weeks Post Molt 1	2	3	6	8	10	12	14
Female	13	N.S.	N.S.	N.S.	N.S.	0.023	0.032	N.S.	N.S.
Male	12	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

1/ Recorded sound of human movement in leaf litter.

2/ V test from J.H. Zar (1974).

3/ Control response was based upon tick behavior in the absence of sound stimuli. Control probabililty was not significant (N.S.).

Table 3

Comparison of the positive directional response of adult *Amblyomma americanum* to sound stimuli[1/] [2/] [3/]

Sex	No. X tested	Weeks Post Molt							
		1	2	3	6	8	10	12	14
Female	13	N.S.	N.S.	N.S.	N.S.	N.S.	0.010	0.021	0.034
	12	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

1/ Recorded sound of human movement in leaf litter.

2/ V test from J.H. Zar (1974).

3/ Control response was based upon tick behavior in the absence of sound stimuli. Control probability was not significant (N.S.).

Table 4

Comparison of response probabilities in three ixodid tick species using two different statistical tests[1/][2/]

Weeks Post Molt[2/]	Sex	Probability[3/] αv	αr
		A. americanum	
10	Female	0.010	0.014
	Male	N.S.	N.S.
12	Female	0.021	0.017
	Male	N.S.	0.027
		A. maculatum	
10	Female	0.032	0.043
	Male	N.S.	N.S.
12	Female	N.S.	N.S.
	Male	N.S.	N.S.
		D. variabilis	
10	Female	<0.0005	0.0011
	Male	0.018	N.S.
12	Female	<0.0005	0.0019
	Male	0.023	N.S.

1/ Rayleigh's test and V test from J.H. Zar (1974).

2/ Ages represent the strongest response period.

3/ αv represents the probability for the critical value V of the V test; αr represents the probability for Rayleigh's critical value r.

Table 5

Directional response of adult Dermacentor variabilis to sound recordings after tarsal removal[1/][2/]

Sex	Weeks Post Molt	Probability[3/][4/] ($\alpha v/\alpha r$) Tarsi I	Tarsi IV
Male	3	N.S./N.S.	N.S./N.S.
Female	3	N.S./N.S.	0.0028/0.0017

1/ Data represent removal of both tarsi.

2/ Removal of one tarsus I resulted in a nonsignificant (N.S.) response.

3/ αv represents the probability for the critical value V of the V test; αr represents the probability for Rayleigh's critical value r. not significant (N.S.) if 0.05.

4/ Control probability was >0.0005/0.001.

Table 6

Directional response of adult <u>Dermacentor variabilis</u> to sound recordings after pretarsal removal[1/]

Sex	Weeks Post Molt	Probability[1/] [3/] (αv / αr) Pretarsi I	Pretarsi IV
Male	8	N.S./N.S.	0.0048
Female	8	0.0031/0.038	0.00061/0.0013

1/ Date represent removal of both pretarsi.

2/ αv represents the probability for the critical value V of the V test; αr represents the probability for Rayleigh's critical value r.

3/ Control probability was $<0.0005/0.0019$.

Table 7

Directional response of adult Dermacentor variabilis to sound recordings of lowered intensity[1/] [2/]

Sex	X No. Tested	Probability[3/] [4/] [5/]	
		αv	αr
Female	15	0.0027	0.0022
Male	14	0.041	N.S.

1/ Sound intensity at center stage = 60 ± 2 dB.

2/ Recorded sound of human movement in leaf litter.

3/ Control response was based upon tick behavior to sound intensity at center stage = 70 ± 2 dB.

4/ αv represents the probability for the critical value V of V test; αr represents the probability for Rayleigh's critical value. α not significant (N.S.) if >0.05.

5/ Control probability was 0.0059/0.0015.

Table 8

Directional response of adult <u>Dermacentor</u> <u>variabilis</u> to different sound source placement[1/]

Sound Source Origin (Degrees)	Weeks Post Molt	Probability[2/] [3/] (αv /αr) Male	Female
Zero	3	N.S./N.S.	.00058/0.0016
180	3	0.0048/N.S.	.0.00062/0.0018

1/ Recorded sound of human movement in leaf litter.

2/ αv represents the probability for the critical value V of the V test; αr represents the probability for Rayleigh's critical value r. α not significant (N.S.) if >0.05.

3/ Control probability was N.S.

Table 9

Induced response of adult *Amblyomma* species to sound recordings[1/]

Hours Desiccation	Weeks Post Molt	Probability[3/] (αv / αr)	
		A. americanum	*A. maculatum*
0	3	N.S./N.S.	N.S./N.S.
1	3	N.S./N.S.	N.S./N.S.
2	3	N.S./N.S.	N.S./N.S.
3	3	0.046	N.S./0.029

[1/] Recorded sound of human movement in leaf litter.

[2/] Desiccation by calcium sulfate at 5 percent R.H.

[3/] αv represents the probability for the critical value V of the V test; αr represents the probability for Rayleigh's critical value r. α not significant (N.S.) if >0.05.

Table 10

Analysis of host habitat recording

Frequency[1/] (Hz)		% of Sample
2500		17.2
2857		10.3
3333		18.1
4000		12.1
5000		17.2
	Total	74.9%

1/ The most preponderant frequencies from a total of 21 ranged from 417-6667 Hz.

Table 11

Directional response of adult *Dermacentor variabilis* to individual frequencies[1/]

Sex	X No. Tested	Frequency (KHZ)	Probability[2/3/3/] αv	αr
Female	11	0.1 - 1.0	0.0058	0.016
Male	10	0.1 - 1.0	0.022	0.034
Female	9	2.0	0.031	0.042
Male	10	2.0	N.S.	N.S.
Female	10	5.0 - 22.0	N.S.	N.S.
Male	10	5.0 - 22.0	N.S.	N.S.

1/ All ticks were 3 WPM.

2/ αv represents the probability for the critical value V of the V test; r represents the probability for Rayleigh's critical value r. α not significant (N.S.) if >0.05.

3/ Control response was based upon tick behavior in the absence of sound stimuli.

4/ Control probability was N.S.

VITA 2

William Donald Bunn

Candidate for the Degree of

Master of Science

Thesis: THE ETHOLOGICAL BASIS OF SOUND AS A HOST DETECTION CUE IN IXODID TICKS

Major Field: Entomology

Biographical:

Personal Data: Born in Mt. Clemens, Michigan, August 27, 1953, the son of Mr. and Mrs. J. E. Bunn.

Education: Graduated from Charleston High School, Charleston, West Virginia, May, 1971; received Bachelor of Science in Agriculture degree from New Mexico State University, Las Cruces, New Mexico, May, 1979; completed requirements for the Master of Science degree in Entomology at Oklahoma State University, Stillwater, Oklahoma, July, 1983.