LABORATORY TESTS OF CANDIDATE CHEMICAL ATTRACTANTS FOR TICKS

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PREFACE

Continued emphasis on environmental protection against persistent toxicants currently in use necessitates more specific means of control against many arthropod pests. The highly developed chemosensory ability of ticks possibly makes them susceptible to target specific toxicants and possibly offers one means of applying such a specific type of control.

A review of the literature revealed that an effective test system for screening candidate tick attractants did not exist and the only previous studies on tick attractants were highly inconsistent. Therefore, the objectives were to develop effective test methods for conducting olfactory attractant studies and to utilize existing methods for purposes of screening large numbers of candidate chemicals. Observations were made on a large group of organic and inorganic chemicals that were selected because of their occurrence in a bio-system and possible influence on tick behavior.

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CHAPTER I

INTRODUCTION

Laboratory evaluation of the influence of chemicals on tick behavior was necessary to provide needed information concerning the stimulating effects of receptors and worthiness of a chemical as an attractant. The studies described herein have been conducted because of the desire to have the techniques for testing large numbers of chemicals to ticks in the laboratory.

The overall attempts to develop methods for survey and control of ticks through chemo-attraction was supported by the Department of the Army Medical Research and Developmental Command. Present studies being conducted include field investigations for trap development and improvement, evaluation of various parameters such as temperature, habitat type, migration and effect of trapping with CO_2.

As emphasized by this brief synopsis of progress in the area of tick chemo-attraction, laboratory investigations such as screening of candidate chemo-attractants become a vital objective if new methods of control are to be developed.

Particular emphasis was placed on the conditions for testing techniques and the methods used. The variability of chemo-sensory testing was recognized by preliminary observations at the O.S.U. tick research laboratories and therefore considerable attention was given to the possible causes of variation and attempts to minimize variability were made.

The basis for selection of chemicals used in these studies was their common occurrence in a biological system, cost and availability, or perviously reported influence on other blood sucking arthropods.

The equipment used in the olfactory studies was designed specifically for ticks since a system for conducting screening of a large group of chemicals was non-existent.

The objectives of this study were to utilize available techniques to screen chemo-attractants and provide a new olfactometer system for conducting tests of the lone star tick's response to the chemicals.

CHAPTER II

RESPONSES OF *AMBLYOMMA AMERICANUM* (L.) (ACARINA: IXODIDAE) TO CANDIDATE CHEMICAL ATTRACTANTS

Host seeking responses of ticks are assumed to be olfactory, tactile, or gustatory; each response accounts for a particular reaction that aids the arthropod in finding a host or final selection of a site for feeding. The evidence that carbon dioxide is a stimulant and attracting substance for certain tick species is well documented. Garcia (1962) reported that *Ornithodoros coriaceus*, *Dermacentor accidentalis*, and *Ixodes pacificus* were attracted to CO_2. Others have demonstrated the effectiveness of CO_2 in traps (Miles, 1968; Wilson, *et al.*, 1972). The latter shows the attraction of all developmental stages of the lone star tick [*Amblyomma americanum* (L.)] to CO_2.

Lees (1947) suggested that palpi of the sheep tick *Ixodes ricinus* L. bear the receptors responsible for detection of an appropriate attachment site once a suitable host is found. These findings support assumptions that this region bears contact receptors, but very little information is available as to the types of chemicals responsible for their stimulation.

Dethier (1957) provided a review of the sensory physiology of ticks which included the report by Totze (1933) that butyric acid was a tick attractant; however, Lees (1948) later found that ticks were either

repelled or indifferent to this chemical.

The purpose of the investigation reported herein was to determine behavioral patterns of ticks exposed to filter paper treated with various candidate attractants.

Materials and Methods

Test Animals and Holding Containers

Ticks used in all experiments were reared on rabbits at the Oklahoma State University Entomology tick research facility. Nymphs were collected at the time of drop-off and placed (300-400) in cartons that were kept at 27°C in humidity chambers with a realtive humidity above 90%, and photoperiod of 14 hr day, 10 hr darkness. Thirty adults were transferred to plastic vials at age 4-6 weeks. Ticks were randomly tested against two sets of chemicals in one week and were not used thereafter.

By these methods only ticks that were of a known age and previously held under similar environmental conditions were used.

Procedures for Handling Ticks and Testing Candidate Materials

The apparatus used for evaluating and screening chemicals for attractants was a turntable 30" in diameter and constructed from 3/4" plywood. The turntable was powered by a 120 V electric motor rotating at 3 RPM. The unit was situated in a small constant temperature (27 $\pm$ 2°C) room, to provide a constant testing environment. Test chambers for measuring the responses of ticks consisted of 90 mm sterile disposable petri dishes, filter papers treated with chemicals to be tested and a glass collar cut from tubing (3.7 cm by 90 mm ID) to

provide a test chamber. Mesh cloth over the top of the chamber provided cover to prevent ticks from escaping (Figure 1, Appendix).

Chemicals to be tested were weighed on an analytical balance on the day of testing and dissolved in an appropriate solvent (H_2O or methanol). Materials were prepared for testing at 0.5 and 0.005%. Test solutions were prepared daily and kept in capped test tubes for short periods until they were used to impregnate the filter paper.

Sterile gloves and disposo-pipettes were used to minimize contamination of filter papers to be treated. Ninety millimeter filter paper discs were treated by saturating one-half of a filter paper with a candidate chemical dissolved in a solvent. The other half of the paper was treated with solvent only and served as a control. Filter papers were air dried at room temperature for one hour before each test.

After treated papers were dried, they were placed in a test unit and 15 ticks/unit were added. Test chambers were situated in the turntable so that the median line was directed toward the center of the board (Figure 2, Appendix). The two concentrations of a chemical were positioned 180° apart. All units were oriented with the chemically treated half of the paper facing the direction of motion during the first 15 minutes. Orientation of the treated surface was reversed during the second 15 minutes. Responses were determined by counting the number of ticks on the treated and untreated sides of the filter paper at the end of each 15 minute period of testing.

The data were analyzed for significance using Duncan's Multiple Range test for comparison of treatment means.

Results

The response of lone star tick adults to chemically treated filter papers is given in Table 1 (Appendix). Of 67 chemicals tested, the greatest affinity was shown for those compounds containing sodium. Of the top five ranked chemicals, only one, (D-(+)-lactose, was not a sodium containing compound. Sodium molybdate was most attractive with a mean of 8.7 and was significantly different ($P \leq .05$) from chemicals 26-67.

Analysis by Duncan's Multiple Range test showed that chemicals 1-25 were insignificantly different in the responses they elicited. Of this group, five were amino acids (L-histidine, L-(+)-lysine, DL-isoleucine, DL-cysteine and DL-threonine). Also included in this group were two sugars, D-(+)-lactose and inositol, eliciting responses of 8.4 and 7.9, respectively. Two compounds containing potassium ranked Nos. 9 and 10, but one of these (10) also contained sodium. The remaining chemicals showing responses above average were histamine, which occurs in all animal tissue, egg albumin (a protein), taurine (a nutrient jelly from beef), iodine, ammonium hydroxide, lead nitrate and glutathion.

The mean responses obtained with chemicals 26-57 suggested little preference by lone star ticks. The means obtained with chemicals 62-67 suggest that these materials repel ticks. It is interesting to note that four of the chemicals in this group are acids.

An AOV was performed on responses on different dates, time of day, and concentration of chemical. Date and time were the greatest source of variation in the control treatment (Table 2, Appendix).

In order to determine possible behavioral patterns elicited by the

solvents used, and to establish any unusual effect due to motion, additional control treatments, in which the experimental papers were treated with only solvent on both sides, were included in each assay. The coefficient of variation and averages are shown in Table 3, Appendix. There was little of servable difference between coefficient of variation for the 2 solvents.

Discussion

Chemicals that elicited the highest positive response in the lone star tick contained sodium. Studies on mosquito behavior indicate that mosquitoes possess contact chemo-receptors that react to water, sugars, salts, and blood (Owen, 1963). The importance of sodium ions was shown in studies of the feeding response in Aedes aegypti, (Galun, 1963). It was demonstrated that in addition to the need for optimum osmotic pressure, Na^+ aided in eliciting the maximum feeding response. Hosei (1959) investigated inorganic salts similar to those found in blood and suggested that a definite response to the compounds occurred. The biological significance of the response to sodium is not known, but may suggest that Na^+ is one factor important in initiating the feeding response and/or the selection of a feeding site.

Several types of receptors are recognized in the lone star tick. Morphological descriptions made by Foelix and Axtell (1971) using scanning and transmission electron microscopy indicate that the ciliary region of tick sensilla possess an unusual ("11 + 0") double-tubule arrangement. A recent description of the fine structure of Haller's organ (Foelix and Axtell, 1972) support Lees' (1948) suggestion that the capsule portion of Haller's organ is an olfactory perceiving region.

The types of sense organs possibly responsible for perception of the chemicals tested in the present study may be similar to those described by Elizahov (1963) while conducting electrophysiological studies of Ixodes persulcalus. The receptors on the distal portion of the forelegs and on the pulvillar surface were responsible for perception. These structures could also account for the repellent action shown by chemicals 62-67.

Fluid ingested by haematophagous arthropods is chiefly blood; however, feeding ticks frequently imbibe only clear fluids in the larval stages (Arthur, 1962). This might suggest some stimuli to initiate feeding other than those associated with blood. Stimulations occurring after exposure to iodine, glutathion, egg albumin, lead nitrate and the Na^+ ion could be indicative of this fact. Glutathion has been previously studied and found to be an inducer of feeding in a soft tick, Ornithodoros tholozani. Glutathion is, however, a constituent of blood, and its effects were suggested to be synergistic (Galun, 1965).

CHAPTER III

AN OLFACTOMETER SYSTEM FOR INVESTIGATION AND SCREENING OF POSSIBLE CHEMO-ATTRACTANTS FOR *AMBLYOMMA AMERICANUM* (L.) (ACARINA: IXODIDAE)

Previous investigations of tick chemical perception indicated that techniques were available for measuring responses of ticks to chemicals, but it was concluded that the observed responses were due mostly to gustatory responses (Burris, *et al.*, 1973). True olfactory responses were not discernible, and very little information pertaining specifically to olfactor reception was obvious. The studies reviewed by Dethier (1955) indicate the lack of information on tick chemoreception.

Because CO_2 has been shown to be an important chemical able to elicit a response in ticks from a distance (Wilson, *et al.*, 1972) and possibly orientation of the tick to a host, its use has advantages in attempts at developing an efficient laboratory olfactory system. The present arguments against use of harmful pest control measures draw attention to the possibilities of utilizing olfactory attractants or repellents to manipulate arthropod behavior in improved methods of control.

Factors affecting the responses of an insect to candidate chemicals are discussed by Beroza (1972); these include rearing, normal biological variations, age of insects, light, temperature, and humidity effects.

A perusal of the literature reveals that a system has not yet been applied to tick biological investigations that could effectively control the above variables and be used as a tool to measure chemical attraction to ticks. The objective of this study was to obtain information toward developing such a device to measure the responses of the lone star tick to candidate chemo-attractants.

Materials and Methods

Tick Rearing

All ticks used in the experiments described below were reared at the O.S.U. tick research lab. Replete larvae and nymphs were collected weekly from hosts and placed in cartons in a humidity chamber that was maintained at 90 ± 2% R.H. and 85°F. The ticks used in these experiments were 4 ± 1 week old adults.

Fifteen hundred ticks/week were transferred to humidified vials prior to the day of testing. Chilling was used to facilitate transfer and the ticks were handled with gloves (aseptic) and forceps only. After being transferred to the vials the ticks were maintained within the rearing room under a constant photoperiod of 14 hr day, and 10 hr night. At the start of a test the ticks were moved to the testing room and placed directly into olfactometers.

The Olfactometer System

Each olfactometer (Figure 3, Appendix) consists of a 2 1/4 x 10 1/2 in rectangular plexiglass box with a separate top that contains an exhaust port. The box is partitioned by a sliding door that is opened at the beginning of each test. This device allowed placement of 15

ticks in each chamber for uniformity. One-fourth in plastic tubing served as hose connectors and was cemented on the top of the exhaust chamber and at the end of chambers 1 and 2 of the plexiglass box.

Gilmont^{R} flowmeters capable of flowrates of 0-1500 ml/min measured the air flowrate. Fine needle valves served to regulate the flow of air supplied by a compressed air tank containing 21% oxygen and 79% nitrogen. Two flowmeters were used on each olfactometer to give a consistent flowrate to each end of the chamber. Vacuum exhaust was provided by a Neptune^{R} No. 3 pump. This was connected to a baffeled chamber to provide an even flowrate from each exhaust chamber.

Tests were conducted using a series of seven olfactometers placed in a constant temperature room which was maintained at 84-90°F. Two 8' bar fluorescent lights were placed overhead to provide a consistent source of light.

Chemical Selection and Preparation

Initially, 48 water soluble chemicals were selected for testing. The basis for selection was their availability, cost, and occurrence in excretion and waste products from a biological organism or a previously reported influence on feeding of blood-sucking arthropods. Each chemical was tested at two concentrations - 0.5 and 0.005 per cent. Prior to the day of testing, 0.25 mg of test chemical was weighed on an analytical balance sensitive to the nearest 0.1 mg. The chemicals were placed in solution by placing the weighed portion into a screw top test tube and pipetting the desired amount of solvent.

Based on information obtained by preliminary dilution experiments, a 5% concentration of CO_2 was selected when CO_2 was the candidate

chemical.

Testing Procedures

Filter paper discs (9 mm) were placed in sterile petri dishes and the appropriate chemical solution was poured over the paper until it was fully saturated. The treated filter paper discs were then transferred to plastic nalgene (75 ml) vials.

The olfactometers were always set up (Nos. 1-7) left to right, and positioned with chamber one nearest the operator (Figure 4, Appendix). Vials that contained the test chemical were numbered to correspond to a randomized chemical code (1-49). Control vials that contained the chemical solvent were attached to chamber two. Responses were measured by making counts at 15 minutes. Tests were then terminated.

A 7 x 7 lattice square was selected for the test design (Cochran and Cox, 1967). This design provided eight repetitions and allowed a total of 49 treatments to be included in each repetition.

Results and Discussion

CO_2 Trials

A 5% concentration of CO_2 (provided by the Puritan Co.) proved to be adequate to stimulate ticks and attract them to one end of an olfactometer. The results of Table 4 show overall means for the individual olfactometer. Treatment means ranged from 17.08 to 19.71. The averages depict a net positive attractancy for numbers above 15.0. The coefficient of variation for this group of data was 35%; however, olfactory studies are recognized to have a higher variance than others involving optical or tactile reactions (Wadley, 1967). These values were slightly

lower than the expected efficiency of the system, but some variation is considered to be correctable and is currently being investigated.

Table 5 refers to the time of day the tests were conducted. It is interesting to note that the first test had a significantly different average than was the average at other times of the day. The possibility of a photo-periodic effect has not been overlooked, but a more acceptable explanation may be that the procedures used in testing need some modification. For example, alcohol remaining in minute pores of the olfactometer from the alcohol bath used for cleaning and drying may have influenced tick ability to properly orient toward the attractant, giving rise to a 10% drop in efficiency after the first test. Another possible cause for variation in the system was the needle valves' slight inability to hold a consistently steady flowrate. This valve has just recently been replaced by an acetylene needle valve.

Four age groups were used in these tests. The differences between averages indicate that age accounts for only a slight amount of the overall variation which occurred (Table 6, Appendix).

The results of Table 7 (Appendix) indicate the greatest amount of variation occurred between blocks within the statistical design. These variations could have been caused by unavoidable differences in handling of the ticks and equipment, photoperiod or a time of day effect.

Several of the requirements for an olfactometer were fulfilled by this system (Hosking, 1934). Preliminary observations revealed that the environment presented to the test animals could have the greatest influence on testing; therefore, two important variables, air flow and moisture, were controlled. Flowrate was reduced to the minimum rate and moisture was introduced by use of distilled H_2O on treated filter paper.

This technique was included because the volatility and absorbability of H_2O is good and may act as a carrier for other molecules (Moncrief, 1970). An obvious advantage of this test system was that a group of 49 chemicals could be completely tested and analyzed in approximately 2.5 weeks by a completely randomized statistical design.

The importance of carbon dioxide as a tick attractant has been shown in field trials (Garcia, 1962a, 1962b; Miles, 1968; Wilson, et al. 1972). This information suggests that CO_2 is an important host chemical that attracts ticks, therefore indicating a need to design lab studies to include CO_2 as a stimulating and attracting chemical for comparative purposes.

Changes in adult population distribution of the American dog-tick Dermacentor variabilis (Say), were attributed to the CO_2 gradient from automobile traffic (McEnroe, 1971). Shortening of the life span was attributed to constant movement caused by CO_2 stimulation.

Neville (1964) conducted studies with various concentrations of CO_2 in the lab and under field conditions, and concluded that the Sand Tampan (Ornithodoros savignyi (Audouin) was stimulated and attracted to a CO_2 source being liberated at 1 liter/min. The implication was that exhaled breath of higher animals is "the factor" responsible for stimulating and attracting the Tampan.

Although considerable controversy surrounds our understanding of the role of CO_2 in mosquito attraction, Kellog and Wright (1962) imply that combinations of CO_2, heat, moisture and visual appearance are enough to provide optimum attraction.

The 5 per cent concentration of CO_2 used in these trials[1] was tested during 392 separate treatments that utilized over 11,000 adult lone star ticks of various ages. It is noteworthy that of the stimulation and orientation responses elicited, only an average of 5 negative responses were recorded for each trial, or less than .01% of the recorded responses to 5 per cent carbon dioxide. This leaves little doubt that a directional response occurred in most instances towards this concentration of CO_2.

Chemical Trials

In order to fully test the olfactometer system's effectiveness, a group of chemicals was selected. The chemicals had previously been tested by other techniques (Burris, et al., 1973) and an indication of their influence on contact receptors was known.

Results of the screening tests for the first group are shown in Table 8, Appendix. Egg albumin and the chemicals containing sodium ranked high in these tests as they did in earlier experiments (Burris, et al., 1973) in which it was thought that gustatory responses were primarily involved. Animal emanations, especially eccrine sweat as suggested by Juno (1956), could provide sufficient concentrations of Na^+ to influence the behavior to ticks. Lactic acid and ammonia, which are also present in the eccrine secretions, proved to be unattractive at 0.5 and 0.005 per cent concentrations. Aprocrine sweat contains larger molecules such as proteins and carbohydrates and such as water soluble albumins similar to those which the ticks responded to in a positive

[1]The chemically treated filter paper discs were replaced by checks treated with dionized H_2O while the CO_2 trials were being conducted.

manner in this study.

Lysine has been reported to be the attractive component of an eluate of bovine plasma, and to be attractive to Aedes stimulans and Culex pipiens (Brown, et al., 1961). The affinity shown to lysine in these tests and earlier studies (Burris, et al., 1973) suggests that it might be an important factor involved in host-seeking behavior of the lone star tick. Other amino acids eliciting above average responses were aspartic acid, 0.5, 0.005, and histidine (0.5). Positive responses were also evoked by lead nitrate, thiamine, sucrose, inositol, trehalose, and sulfosalicylic acid.

Chemicals that consistently demonstrated the highest average responses were CO_2 (21.48, Table 5 and 20.65, Table 9 - Appendix), sodium containing chemicals, amino acids and sugars. Although several responses to chemicals approached the averages elicited by CO_2, none of the chemicals tested caused a visual "excitatory" response or stimulated the ticks to activity as strongly as CO_2. The "excitatory" behavior of the tick is impossible to quantitate and remains a weakness of the present olfactometer system. However, the range of 20.65-4.31 of measured responses in Table 9 indicates that the system was somewhat successful in separating chemicals as to their ability to elicit positive attractancy responses.

Since the responses were measured by counts of congregated ticks, those responses evoked by the sodium containing chemicals, proteins, and the sugars are suspected to have acted more as an arrestant than as an attractant. This is also evidenced by the observed lack of "excitatory" behavior of the experimental animals as compared to that caused by CO_2.

The effects of chemical concentration in chemo-attraction studies are discussed by Dethier (1947). Several chemicals, i.e., histidine and sulfosalicylic acid, had caused different responses by ticks at different concentrations. On the other hand, sodium containing chemicals elicited like responses (sodium barbitol, sodium molybdate, sodium succinate, sodium phosphate monobasic) at both concentrations. Any chemical not exhibiting the property of being attractive in concentrations ranging from low to high would have to be excluded as a distance lure in trapping devices.

CHAPTER IV

SUMMARY

Contact and olfactory responses of the lone star tick to candidate attractants were tested against 67 inorganic or organic chemicals soluble in H_2O or methanol. Those chemicals containing sodium appeared to be the most attractive. The least affinity was shown to stearic acid, palmitic acid, oxalic acid, indole, riboflavin and oleic acid. Statistical analysis showed that of the chemicals eliciting the best responses, 5 were amino acids. Because of the types of chemicals eliciting a response and the behavioral patterns observed, the responses are believed to have been caused after stimulation of contact receptors.

Olfactory experiments were conducted in a series of 7 rectangular olfactometers. A 7 x 7 lattice design was used for statistical analysis of the experiments. Extensive trials were conducted by utilizing a 5 per cent concentration of CO_2 to determine the behavior of ticks to this gas in the olfactory system. The results suggest an attracting response was elicited by 5 per cent CO_2, but the averages were lower than the expected efficiency of the olfactometers. The probable causes were considered to be technical problems that are inherent with these types of tests. Chemicals used had been previously exposed to ticks by a different test system and some indication of their activity was known. The results are shown in Tables 8 and 9 (Appendix). Egg albumin, the sodium chemicals, and CO_2 continued to elicite the highest responses.

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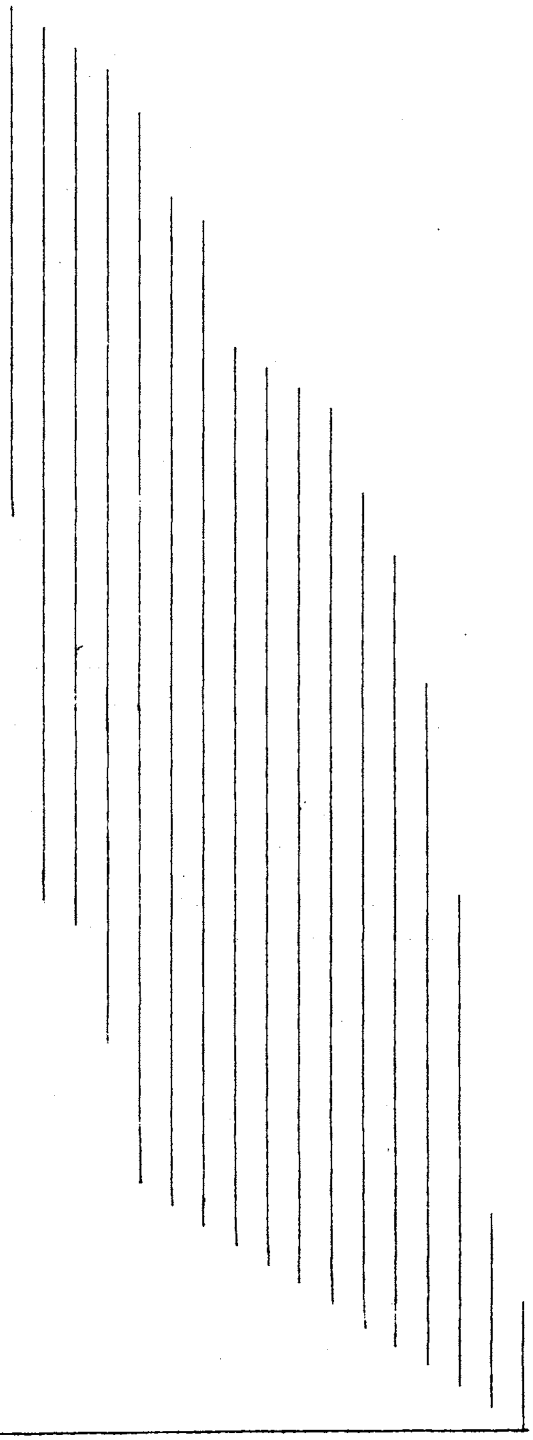
APPENDIX

Table 1. A comparison of the mean average response elicited by chemically treated filter paper to the lone star tick.*

	Chemical	$\bar{X}$
1	Sodium molybdate	8.7
2	Sodium chloride	8.6
3	Sodium sulfate	8.5
4	Sodium bicarbonate	8.4
5	D-(+)-lactose	8.4
6	Sodium barbitol	8.3
7	Iodine	8.3
8	L-histidine	8.3
9	Potassium phosphate (dibasic)	8.3
10	Potassium sodium tartrate	8.2
11	Lead nitrate	8.2
12	Sodium carbonate	8.2
13	Sodium succinate	8.2
14	Egg albumin	8.1
15	L-(+)-lysine	8.1
16	Taurine	8.1
17	Dl-isoleucine	8.1
18	Ammonium hydroxide	8.0
19	Inositol	7.9
20	Sodium phosphate (dibasic)	7.9
21	Glutathion	7.8
22	Histamine	7.8
23	Dl-cysteine	7.7
24	Dl-threonine	7.7
25	Thiamine	7.7
26	Phenol	7.7
27	Glycine	7.6
28	Dl-aspartic acid	7.6
29	Aluminum sulfate	7.6
30	Soy hydrolysate enzyme	7.6
31	Ammonium chloride	7.6
32	Dl-serine	7.6
33	D(-) fructose	7.5
34	Sucrose	7.5
35	Cholesterol	7.5
36	L-proline	7.5
37	L-arqine	7.5
38	Maltose technical	7.5
39	Sulfosalicylic acid	7.5
40	Urea	7.4
41	L-leucine	7.4
42	Potassium phosphate (dibasic)	7.4
43	Glycogen	7.4
44	Isotin	7.3
45	Sodium phosphate (monobasic)	7.3
46	L-glutamine	7.3
47	L-tyrosine	7.3
48	Dl-methonine	7.3
49	Trehalose	7.3
50	Sodium bisulfate	7.3
51	Thymol	7.2
52	Tributyrin	7.2
53	Brewers yeast	7.2
54	Dl-alanine	7.2
55	L-phenylalanine	7.2
56	Lavric acid	7.1
57	L-(-)-tryptophan	7.1
58	Dl-valine	6.9
59	L-(+)-cysteine hydrochloride	6.8
60	Sodium acetate	6.8
61	Yeast hydrolysate enzyme	6.7
62	Stearic acid	6.6
63	Palmitic acid	6.5
64	Oxalic acid	6.4
65	Indole	6.2
66	Riboflavin	5.9
67	Oleic acid	5.6



*Means ($\bar{x}$) followed by a common verticle line are not significantly different at the $P<0.05$ level, Duncan's Multiple Range Test.

Table 2. Analysis of variance for methanol and water variable counts.

Source	DF		Sum of Squares		Mean Square	
	Methanol	Water	Methanol	Water	Methanol	Water
Date	18	36	100.11	264.72	5.56	7.35
Time	1	1	0.05	3.75	0.05	3.75
Date*Time	18	36	61.56	129.53	3.42	3.59
Residual	114	490	506.25	2266.83	4.44	4.62
Corrected Total	151	563	667.99	2264.85	4.42	4.73

Table 3. Distribution pattern of lone star tick adults on solvent treated filter papers in turntable test chambers.

Solvents	N	$\bar{X}$ on treated side	C.V.
*Methanol	151	7.50	28.07
*Water	563	7.72	27.84

*The greatest source of variation occurring for methanol and water was date.

Table 4. Uniformity trial with 5% CO_2 showing the mean number of ticks attracted by plot.

*Plot	N	$\bar{X}$
1	56	19.01
2	56	18.94
3	56	19.71
4	56	17.12
5	56	17.08
6	56	17.94
7	56	18.75

*Plot designates the olfactometer number 1-7.

Table 5. Uniformity trial with 5% CO_2 showing the mean number of ticks attracted by block.

*Block	N	$\overline{X}$	Time
1	56	21.48	8:30
2	56	17.64	9:30
3	56	17.46	10:40
4	56	18.44	12:00
5	56	18.23	1:00
6	56	17.10	2:00
7	56	18.21	3:30

*Block designates the number of times the olfactometers (1-7) were set up.

Table 6. Uniformity trial with 5% CO_2 showing the mean number of ticks attracted by age.

Age	N	$\overline{X}$
08 29	98	17.84
09 07	98	18.32
09 08	98	18.85
09 09	98	18.44

Table 7. Analysis of variance of uniformity trials with 5% CO_2 concentration.

Source	DF	Sum of Squares	Mean Square
Age	3	50.86	16.95
Block	6	709.98	118.33
Age*Block	18	327.86	18.21
Plot	6	340.09	56.68
Age*Plot	18	254.90	14.16
Block*Plot	36	558.65	15.51
Age*Block*Plot	108	1249.48	11.56

Table 8. Influence of candidate attractants on tick behavior during screening trials.

Chemical	Concentrations	Mean Number of Ticks Responding to the Treatment After 15 Minutes Exposure
Egg albumen	.5	19.00
	.005	18.50
Sucrose	.5	18.37
	.005	15.58
Sodium sulfate	.5	17.87
	.005	
Sodium barbitol	.5	17.25
	.005	17.00
L (+) lysine dihydrochloride	.5	17.25
	.005	17.48
DL - aspartic acid	.5	17.12
	.005	15.07
Lead nitrate	.5	16.87
	.005	13.88
Sodium molybdate	.5	16.87
	.005	16.32
Thiamine hydrochloride	.5	16.62
	.005	15.92
Sodium succinate	.5	16.50
	.005	14.84
Inositol	.5	16.37
	.005	14.44
Sulfosalicylic acid	.5	16.37
	.005	19.48
Trehalose	.5	16.37
	.005	14.82
Lactic acid	.5	16.25
	.005	12.29
L-histidine	.5	16.25
	.005	11.19

Table 8. Continued.

Chemical	Concentrations	Mean Number of Ticks Responding to the Treatment After 15 Minutes Exposure
Sodium acetate	.5	16.25
	.005	12.57
D (-) fructose	.5	16.12
	.005	14.38
Potassium phosphate (monobasic)	.5	16.00
	.005	14.44
Sodium bicarbonate	.5	15.87
	.005	12.71
D (+) lactose	.5	15.00
	.005	15.13
DL-valine	.5	15.00
	.005	13.05
Maltose technical	.5	14.87
	.005	14.56
Sodium carbonate	.5	14.87
	.005	16.31
L (+) cysteine hydrochloride	.5	14.62
	.005	14.07
Sodium bisulfate	.5	14.62
	.005	14.83
Aluminum sulfate	.5	14.37
	.005	13.75
Ammonium hydroxide	.5	14.37
	.005	15.50
Potassium sodium tartrate	.5	14.25
	.005	15.09
Sodium phosphate (monobasic)	.5	14.12
	.005	14.20
Soy hydrolysate enzyme	.5	14.12
	.005	13.06
Sodium phosphate (dibasic)	.5	14.12
	.005	12.02

Table 8. Continued.

Chemical	Concentrations	Mean Number of Ticks Responding to the Treatment After 15 Minutes Exposure
L-phenylalanine	.5	13.87
	.005	13.42
Carbon dioxide	.5	13.25
	.005	10.64
DL-threonine	.5	13.00
	.005	14.29
Potassium phosphate (dibasic)	.5	13.00
	.005	12.55
Glycogen	.5	12.75
	.005	12.29
L (-) arginine	.5	12.75
	.005	12.29
Urea	.5	12.75
	.005	14.23
Dl (-) alanine	.5	12.62
	.005	11.93
Sodium chloride	.5	12.62
	.005	14.25
Brewers yeast	.5	12.25
	.005	13.39
Yeast hydrolysate enzyme	.5	12.25
	.005	14.78
Glycine	.5	11.87
	.005	15.72
L (-) leucine	.5	11.87
	.005	15.66
Ammonium chloride	.5	11.75
	.005	12.62
DL (-) isoleucine	.5	11.75
	.005	15.51
DL (-) methionine	.5	11.75
	.005	14.30

Table 8.

Chemical	Concentrations	Mean Number of Ticks Responding to the Treatment After 15 Minutes Exposure
Glutathione	.5	11.12
	.005	10.71
Taurine	.5	10.87
	.005	14.32

Table 9. Influence of candidate attractants on tick behavior during screening trials.

Chemical	Concentration	Mean Number of Ticks Responding to the Treatment After 15 Minutes Exposure
Carbon dioxide	.5	20.65
L - (-) - tryptophan	.5	19.15
Potassium chloride	.5	17.91
Citric acid	.5	17.89
Hemoglobin	.5	17.41
Glutamic acid	.5	17.18
Oxalic acid	.5	16.96
Gelatin hydrolysate	.5	16.57
Sodium metabisulfite	.5	16.56
Guanidine hydrochloride	.5	16.43
Amino acetic acid	.5	16.00
DL amino n butyric acid	.5	15.45
Lactalbumin hydrolysate	.5	15.33
L - glutamine	.5	14.83
Raffinose	.5	14.83
1,2,3, triketohydrindene	.5	14.54
Iodine	.5	14.30
3 methyl indole	.5	14.28
L - proline	.5	14.27
Sodium citrate	.5	14.13
Methanol (absolute)	.5	14.10
Ethanol	.5	14.10
Potassium sulfate	.5	13.95

Table 9. Continued.

Chemical	Concentration	Mean Number of Ticks Responding to the Treatment After 15 Minutes Exposure
Potassium iodide	.5	13.80
Histamine	.5	13.66
DL-serine	.5	13.64
Lauric acid	.5	13.53
DL alanine	.5	13.47
Calcium hydroxide	.5	13.09
Clyclohexane	.5	12.87
Phenolphthalein	.5	12.83
Silicic acid	.5	12.57
Uracil	.5	12.32
Isatin	.5	11.99
Stearic acid	.5	11.87
Calcium carbonate	.5	10.99
Tributrin	.5	10.84
Thymol	.5	10.81
Palmitic acid	.5	10.71
Casein hydrolysate	.5	10.62
Glyoxal	.5	10.58
2 amino, 2 (hydroxy methyl) 1-3 propanedial	.5	10.50
Calcium chloride, anhydrous	.5	10.47
Inulin	.5	10.42
Oleic acid	.5	10.01
Cholesterol	.5	9.58

Table 9. Continued.

Chemical	Concentration	Mean Number of Ticks Responding to the Treatment After 15 Minutes Exposure
Pyridine	.5	9.31
Indole	.5	9.12
Cyclopentanone	.5	4.31

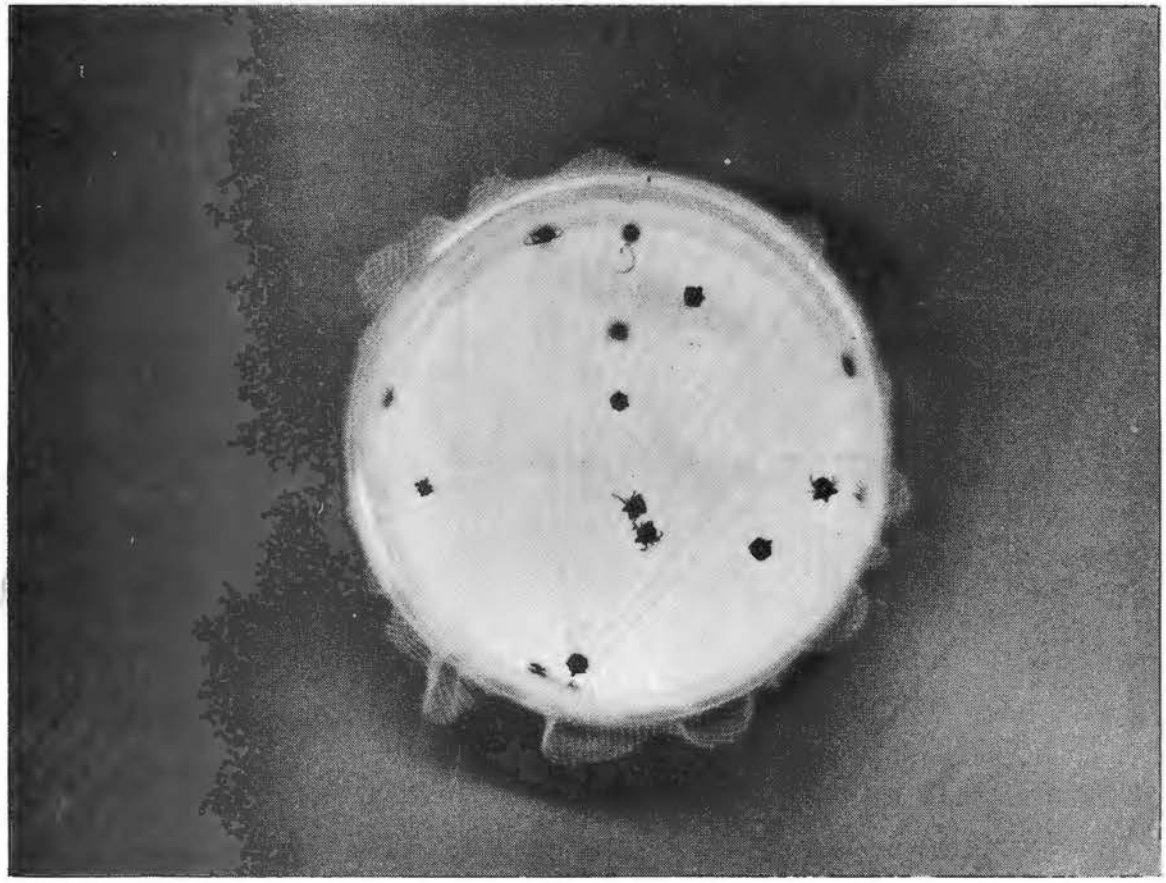


Figure 1. Test chambers used for measuring the responses of ticks consisted of sterile petri dishes, treated filter paper and mesh cloth.

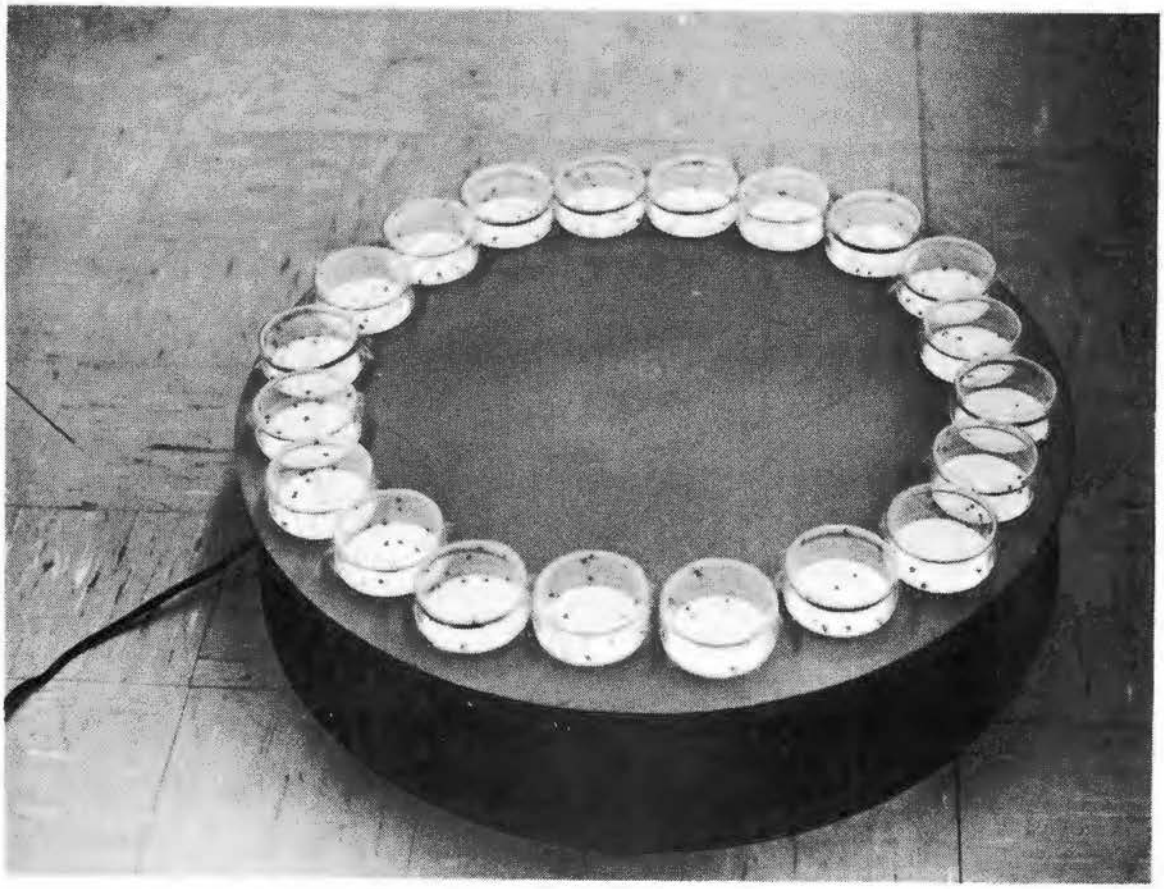


Figure 2. The test chamber arrangement on a turntable during a test.

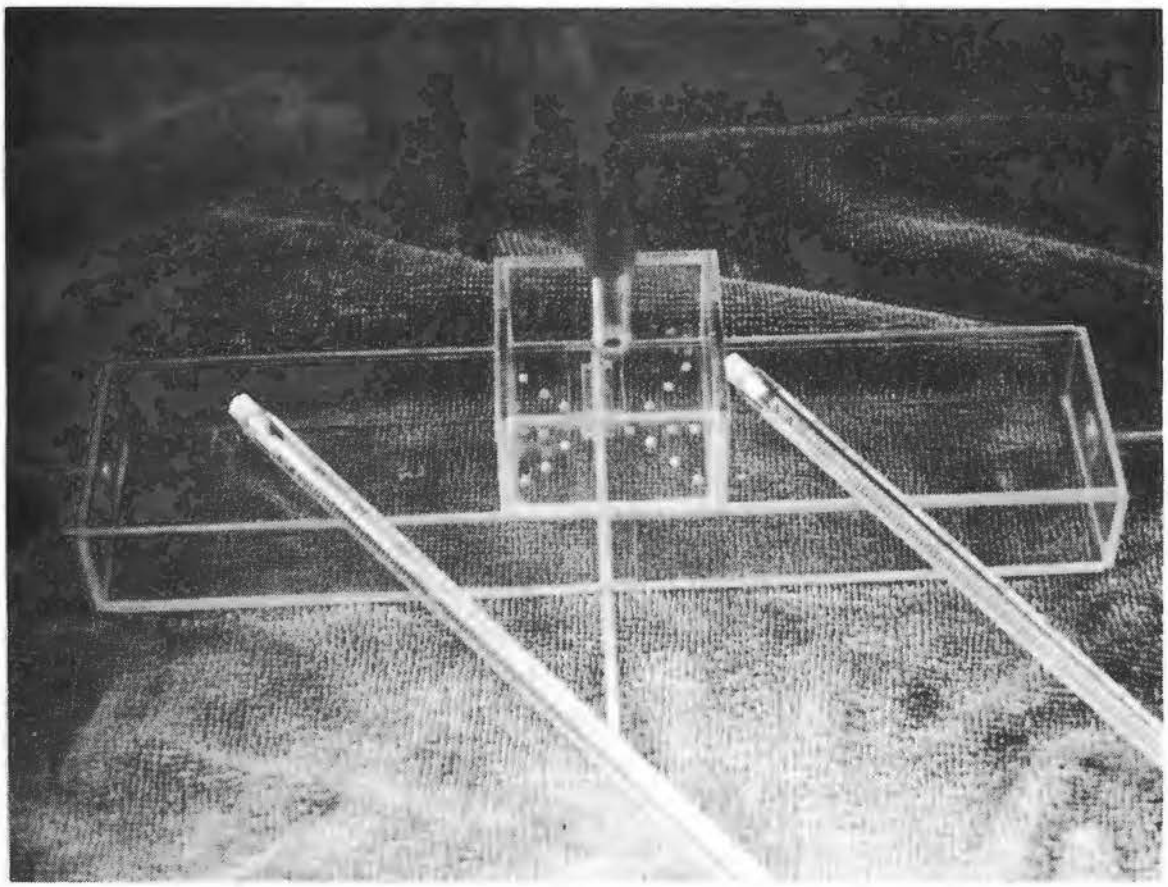


Figure 3. Each olfactometer consisted of a 2 1/4 x 10 1/2 inch rectangular plexiglass box with a separate top that contained an exhaust port. Also shown are two flowmeters.

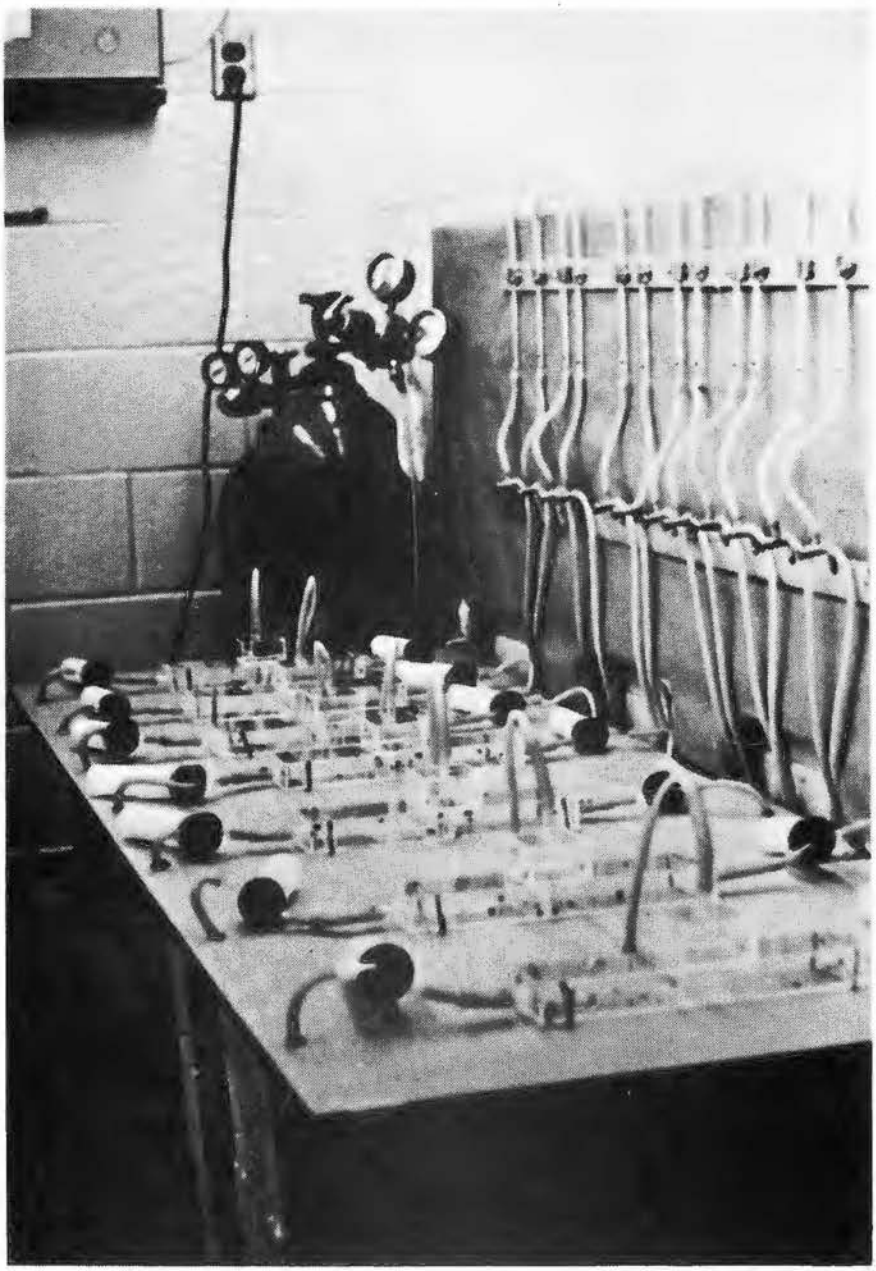


Figure 4. The appearance of the olfactometer system during tests.

VITA 2

Eugene Burris

Candidate for the Degree of

Master of Science

Thesis: LABORATORY TESTS OF CANDIDATE CHEMICAL ATTRACTANTS FOR TICKS

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