

SENSORY DISCRIMINATION OF CHEMICALS BY

Periplaneta americana (L.)

By

Donald M. McCroddan

Bachelor of Arts

John Brown University

Siloam Springs, Arkansas

1961

Submitted to the Faculty of the Graduate School
of the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
August, 1963

JAN 8 1964

SENSORY DISCRIMINATION OF CHEMICALS BY
Periplaneta americana (L.)

Thesis Approved:

Deatonell

Thesis Advisor

Robert A. Morrison

Chas. F. Zenserson

Arvid M. Heston

Dean of the Graduate School

542085

PREFACE

After conferring with Dr. D. E. Howell, Professor and Head of the Department of Entomology, the author decided that a thesis problem concerning the sensory discrimination of chemicals by Periplaneta americana would be interesting, challenging, and practical.

The author wishes to express appreciation to his major adviser and committee chairman Dr. D. E. Howell for his encouragement, interest, guidance, and thoughtful constructive comments during the research work and the preparation of this manuscript. Appreciation is expressed to Dr. Robert D. Morrison, Professor of Mathematics, for his interest, encouragement, and advice on the statistical methods involved, and preparation of this manuscript, and to Mr. Charles F. Henderson, Professor of Entomology for his constructive comments during the preparation of this paper. Special appreciation is expressed to Dr. Lyle D. Goodhue, Manager, Agricultural Chemicals, Research Section, and other personnel of Phillips Petroleum Company for their interest and cooperation which has helped make this project possible, and for the supply of selected chemicals used in the research.

Special appreciation is also expressed to my wife Denise, for constant encouragement, helpful suggestions, and typing during the preparation of this paper.

TABLE OF CONTENTS

	Page
INTRODUCTION.	1
REVIEW OF LITERATURE.	2
Chemical Orientation	2
Common Chemical Sense.	4
Test Insects	4
Response to an Attractant.	5
METHODS AND MATERIALS	7
Test Insects	7
Techniques	7
Measurements	8
Tube Filling	8
Accepted Experimental Unit Construction.	8
Unit Preparation for Testing	11
Preliminary Test Unit Checks	11
Chemical Preparation	11
Data Analysis.	13
RESULTS AND DISCUSSION.	15
Experimental Unit.	15
Preliminary Test Unit Checks	16
Chemical Preparation	16
Chemical Testing	17
Experimental Limitations	18
Test Insects	18
Concentration Effect	19
SUMMARY AND CONCLUSIONS	20
REFERENCES CITED.	22
APPENDIX.	24

LIST OF TABLES

Table	Page
1. Chemicals which attracted or repelled <u>P. americana</u> during the screening tests	12
2. Mean consumption of Chemicals 2217 and 3329 at several concentrations.	25
3. Mean consumption of ethyl alcohol at several concentrations . .	25
4. Mean consumption of chemicals 2269, 3381 and 2271 at several concentrations.	26
5. Mean consumption of chemicals 3367, 3369, and 2259 at several concentrations.	26
6. Mean consumption of chemicals 2321, 2227, and 3343 at several concentrations.	27
7. Mean consumption of chemicals 3225 and 2117 at several concentrations.	27
8. Mean consumption of chemicals 2229, 3351, and 3353 at several concentrations.	28
9. Mean consumption of chemicals 3363, 2253, and 3365 at several concentrations.	28
10. Mean consumption of chemicals 3371, 2261, and 3373 at several concentrations.	29
11. Mean consumption of acetone at several concentrations	29
12. Mean consumption of ethyl alcohol at several concentrations . .	30
13. Mean consumption of the chemicals found to be attractant to <u>Periplaneta americana</u>	30

LIST OF FIGURES

Figure	Page
1. Components of experimental unit showing plastic cylinder, assembled unit, and cover to exclude light.	10
2. Tubing assembly showing arrangement of consumption and evaporation check tubes.	10
3. The analysis of variance form used when preparing the raw data for interpretation.	14

INTRODUCTION

Each year the role played by cockroaches in disease transmission becomes more and more evident. Cockroaches have been experimentally shown capable of acquiring bacteria by crawling over contaminated cultures and depositing them on contacted food materials (Herms and Nelson 1913). Included in the list of pathogenic organisms isolated from wild cockroaches are several strains of the poliomyelitis virus, about forty species of bacteria mostly associated with enteric diseases, the protozoan Entamoeba histolytica (Schaudinn) responsible for amoebic dysentery, and many others. Because of omnivorous habits, feeding on most human foods as well as human excrement, blood, dead insects, and many other materials, it is easy to recognize the hazard involved as these insects move about. The economic problem presented emphasizes the need for sound control methods.

An objective of this research was to determine and evaluate the reactions of P. americana to certain liquid chemicals in an effort to recognize materials with attractant properties that might be used in a bait.

This work also included the development and testing of an experimental method used in an effort to accomplish the above objective. The cockroaches were subjected to different chemical concentration levels using water as a check, and the resulting data were analyzed statistically.

REVIEW OF LITERATURE

It has long been man's problem to find ways of combating the insects which have continually challenged him for his food, clothing, home, and freedom from personal annoyance,

Crude, naturally occurring repellents were the first ones used to find relief, but not until the end of the nineteenth century were any produced commercially. The use of attractants for control was still not recognized at that time, and presently most knowledge concerning these two means of control deals with repellents. Dethier (1947) reviews the earlier major contributions made to these fields.

The trend of control research today is towards the understanding of chemoreception or the physiological processes occurring in some sensory receptor cells. Research concerning the sensory system of insects has followed two trends, the empirical approach, and that directed towards an understanding of the basic physiology of chemoreception (Hodgson, 1953). The latter should ultimately prove to be the most important, but the empirical method will help meet the need for control materials today.

CHEMICAL ORIENTATION. Insect orientation depends on a complex array of stimulation. For example, light, heat, humidity, and gravitational forces affect behavioral patterns in most insects, but perhaps the more important governing stimuli are chemical in nature (Dethier, 1957a). A chemical stimulus may act as an attractant, directing an insect over long distances, or to release innate behavioral patterns. However, no one attractant alone guides an insect to a habitat, mate, or food

(Dethier, 1947). Odorous stimuli may act as attractants and releasers, while those thought of as taste or contact stimuli, act primarily as releasers (Dethier, 1957a). Acting as a releaser, a stimulus may induce feeding, mating, oviposition, and other behavioral patterns.

Odorous and gustatory stimulants may cause insects, which have happened along by chance, to stop exploratory movements. Stimulants with this characteristic are known as "acceptants" or "arrestants" (Dethier et al. 1960). The resulting accumulation of insects, in contrast to that due to the influence of an attractant, is not the result of insects pulled in from a widespread population.

Dethier (1957a) states that odor initiates stream orientation, that insects orient to an odor source better in a current than in still air, but that removal of the antennae prevents orientation, and that insects with a single antenna are still capable of orientation either in still air or in a current.

Green et al. (1960) define an attractant as a material whose vapor, upon reaching the olfactory or other receptors of an insect, will cause an approaching response. A repellent is defined by Dethier (1960) as "a chemical which causes insects to make oriented movements away from its source". He also states that chemostimuli emanating from distant sources are said to be smelled while those originating from sources in close contact with the insects (within the buccal cavity, or touching the legs, palpi, or antennae) are said to be tasted. He also pointed out that taste stimuli do not permit directive locomotor responses, but both odor and taste stimuli may be repellent. Diametrically opposed reactions may be produced by two substances differing in concentration alone.

Failure to consume a material may be due to absence of an attractant

to trigger a reaction, to an olfactory repellent preventing initial feeding, to contact repellents preventing or reducing feeding after sampling, or to toxic materials which, while they might not kill the insect, cause it to cease feeding or move to another spot (Dethier, 1947).

COMMON CHEMICAL SENSE. According to Dethier and Chadwick (1948), certain chemicals act upon a common chemical sense, but for excitation, a high concentration of stimulus is necessary and the response is always negative or avoiding in nature. Using behavioral and electrophysiological methods, Roys (1954) showed that repellent vapors of some compounds act upon the legs (not known to bear olfactory receptors) and even on the isolated nerve cords of cockroaches. He later found (1956) that nerve fibers and neurons in the tarsi and ventral nerve cord responded to direct applications of salt, acid, sucrose, or quinine in concentrations as low or lower than the concentrations which have been reported as normal taste thresholds in behavioral studies. This suggests that if chemoreception of this sort is a fundamental property of nerve tissue, then no special receptor is needed to translate a chemical action into nerve impulses, or that location rather than specialization determines the function of a receptor. Price (1963) found that even though the antennae had been removed, P. americana were still able to sense the presence of repellents. This may confirm Roys' work or suggest other receptor locations. In contrast to this, Wharton et al. (1954a) found that male P. americana lost all response to the powerful sex attractant emitted by the female when only the antennae were amputated.

TEST INSECTS. Sun (1960) conducted research to determine the effects of pretest conditions on insect insecticide reaction tests. He found that humidity, temperature, diet, and state of starvation caused some variation

in test results. He also found that stage of development, age, and sex affect extent of variation. Starved insects produce less variable results, evidently because of a more nearly uniform metabolic process. Dethier and Chadwick (1948) recognized that by far the most important factor affecting acceptance thresholds is the nutritive state of the animals, and it is a common observation that acceptance thresholds fall when insects are maintained continuously on a water diet or are otherwise subjected to complete or partial starvation.

Wharton et al. (1954b) found that three days were apparently sufficient for male P. americana to return to normal after the application of toxic paint for identification. This conclusion was based on reactions to established test procedures.

RESPONSE TO AN ATTRACTANT. Wharton et al. (1954a) found that isolated cockroaches were not suitable for testing, evidently because of their gregarious habits. Upon exposure to the attractant, those cockroaches which reacted were observed to show sudden alertness recognized by straightening of the legs or slight raising of the body, to rapidly move their antennae, and to search for the stimulant source.

It was found that cockroaches may be highly sensitive for a few days, weeks, or months and then become quiescent gradually or suddenly. However, in spite of the variability in response, similar groups of cockroaches from the same colony could be depended upon to behave alike under comparable conditions.

Wharton et al. also conducted tests to determine the responses to repeated tests using the same attractant concentrations. They found a decline in percent response true to all levels tested. It was also demonstrated that the decline became more and more significant as higher

concentrations were tested. The data suggested a progressive decline in percent of cockroaches responding to an attractant concentration. This decline was hypothesized to be nervous system fatigue.

METHODS AND MATERIALS

TEST INSECTS. Colonies of P. americana maintained in the laboratory were fed Purina Dog Chow and supplied with water. Only adults were used and no attempt was made to regulate the number of each sex used when testing.

TECHNIQUES. Several experimental designs were tested to determine a satisfactory means of evaluating the reactions of P. americana to a variety of liquids. The original design consisted of perpendicularly suspended glass tubes inside a 16- by 8.5- by 9-inch aquarium. The lid held 30 tubes in 3 longitudinal rows of 10 each, the bases of the tubes extending to 0.8 inch from the inside lower aquarium surface. The tubes were closed at the top by a short length of rubber tubing and a screw-type clamp, and were held at the proper distance from the aquarium base by a narrow band of rubber tubing. Data were obtained by measuring the liquid level change due to evaporation and consumption by the cockroaches. Evaporation check tubes were also present for each of the liquids being tested. Small screen cages were placed over the lower ends of these tubes to prevent feeding and allow for measurement of evaporation rates. Each evaporation rate was later subtracted from the liquid level change in the corresponding consumption tubes to establish the consumption rate.

From this point "consumption tubes" will refer to those tubes from which liquid could be consumed. "Evaporation check tubes" will refer to those tubes fitted with a screen cage on the lower end to prevent liquid consumption.

MEASUREMENTS. Measurements were made by holding a millimeter ruler next to each tube and observing the distance from the liquid level to the original level mark.

TUBE FILLING. Tubes were filled by sucking the test liquid above a black mark on the tube and then allowing the level to fall to the mark by manipulation of the top screw clamp. The suction device used was a pipette filler.^a The lower end of the tube was then wiped off with a piece of cotton soaked in acetone to prevent supporting frame contamination as much as possible.

This equipment was discarded because of greater fluid consumption from tubes near the corners than in other locations when all tubes contained the same liquid.

ACCEPTED EXPERIMENTAL UNIT CONSTRUCTION. To eliminate the problem of positional affect, a cylindrical container and circular arrangement of tubes were tried and accepted. For the basic container, a 25-gallon shortening can, cut off to an inside height of 9 inches, was tried. A disadvantage of limited observation of the roaches and their reactions was quickly recognized, and a more suitable container was sought.

Cylinders 16 inches in diameter and 9 inches tall were constructed from sheets of cellulose nitrate. Around the top and bottom were placed stiff wire loops to add rigidity to the thin clear plastic. Each cylinder was placed on a baseboard of 0,5-inch, three-ply interior plywood, but the two were not fastened together to allow convenient changing of the baseboard paper cover. The lids, 17.5 inches in diameter, were cut from the same type plywood as that of the baseboards and each had a 9.75-inch

^aFisher - propipette

circular hole in the center covered with nylon tulle to allow ventilation.

Eight-millimeter glass tubing, with an inner diameter of 6 mm, was used for the consumption and evaporation check tubes. These tubes were arranged in two rings, an outer ring of 13.25 inches in diameter consisting of 24 evenly-spaced consumption tubes, and an inner ring of 11 inches in diameter consisting of 12 evenly-spaced evaporation check tubes. The lid tube holes were 0.3 inches in diameter.

Because of tube alignment difficulties, it was necessary to construct a light wire framework to stabilize the tubes. This frame, placed about 3 inches below the lid, consisted of 4 loops, 1 for the inside and outside of each ring of tubes, with short lengths of wire soldered across to stop lateral movement. The wire used was 0.045-inch music or piano wire sold in many hobby shops.

The small wire cages used on evaporation check tubes were constructed from thin copper tubing with an inner diameter of 8.3 mm, and 16- by 16-mesh wire screening. One and one-fourth inch lengths of the tubing were cut and 1.75-inch cylinders of the screening were soldered to the lower three-fourths of the tubing. The end of the screen cylinder away from the copper tube was then pinched shut to complete the cage. Two opposing slits were cut in the exposed end of the tubing to hold a wire pinch clamp which secured the cage to an evaporating check tube. The pinch clamps were made from the same wire used to construct the glass tube stabilizers.

Since P. americana is greatly affected by light conditions, a cardboard box was placed over each complete unit and a black cloth band secured around the base to keep out light.



Fig. 1. Components of experimental unit showing plastic cylinder, assembled unit, and cover to exclude light.



Fig. 2. Tubing assembly showing arrangement of consumption and evaporation check tubes.

UNIT PREPARATION FOR TESTING. Approximately 35 adult cockroaches were used in each unit, and those injured due to close confinement were replaced.

Before each test was begun, the newspaper covering the baseboard was replaced with at least three fresh layers. Over this was placed a piece of plain brown paper to provide a uniform lower surface. The tubes and other glassware were cleaned in a solution of sulfuric acid saturated with potassium dichromate to remove any trace of chemical remaining from a previous test, as well as the characteristic cockroach odor.

PRELIMINARY TEST UNIT CHECKS. After the experimental unit construction had been completed, statistical evaluation was made of the evaporation rates for the tubes in the inner and outer circles and also from position to position around the circles. Ten tubes, all containing water and fitted with evaporation check cages, were placed in each circle. The evaporation rates were recorded at 24-hour intervals for 2 days. A single evaporation rate average was used in calculating consumption rates.

CHEMICAL PREPARATION. Most of the chemicals tested were received as 5 percent solutions from Phillips Petroleum Co., Bartlesville, Oklahoma. The solvents were either water, acetone, alcohol, or combinations of these, some with the emulsifier Atlox 1045-A added. The chemicals chosen for this research had demonstrated attractant qualities to either cockroaches or other insects in previous research. Chemicals which attracted or repelled P. americana in this research are listed in table 1.

Before the testing was begun, alcohol and acetone were tested in

Table 1.--Chemicals which attracted or repelled P. americana during the screening tests.

Chemical Number	Physiological Reaction ^a	Chemical Name
2217	A	5-(4-Pyridyl-N-oxide)isothiuram chloride
2227	A	1-Glutamic acid
3369	A	2-Acetamidopyridine
2269	A	2-Amino-4chloro-6-methylpyrimidine
	A	Ethyl Alcohol
3343	R	4,4'-Dipyridyl disulfide-N,N'-dioxide
3351	R	2-Amino-4,6-dimethylpyridine
3353	R	2-Amino-6-hydroxypyridine
2117	R	Isonicotinic thioamide
3365	R	4-Acetamido-5-methyl-2,6-diethylpyrimidine
3367	R	N-Formylpiperidine
2259	R	4-Nitro-N-oxypyridine
3373	R	1-Nitroso-2-methyl-5-ethylpiperidine
3381	R	4,6-Diamino-5-nitropyrimidine

^aAttractant and repellent are represented by "A" and "R" respectively.

the unit to determine if either of these chemical solvents were attractant or repellent in the range of concentrations used in testing.

Chemicals were tested for attractant and repellent properties at concentrations from 0.1% to 0.00001%, reducing the concentrations ten-fold at a time. Thus, at the commencement of testing, five concentrations of two chemicals were tested per unit. At a later date, it was decided that five different concentrations in that range were not necessary to isolate a chemical with attractant properties, but that three selected concentrations would be sufficient. At the start of testing, two chemicals, each in five concentrations, were tested against water as a check in each experimental unit. In the outer consumption ring of 24 tubes, each concentration was replicated twice; in the inner evaporation ring of 12 tubes, each concentration was represented once. Water was placed in the remaining four tubes in the outer ring and in the two remaining tubes in the inner ring. When the change to three concentrations for each chemical was made, three chemicals were tested in in each unit, the remaining tubes being filled with water.

After screening of the chemicals had been completed, and five with attractant properties had been recognized a test was set up to compare the five. In this experiment each chemical was tested at two concentrations, the one that had shown attractant characteristics in previous tests, and the next ten-fold concentration either above or below.

DATA ANALYSIS. During each test, results were recorded at 24-hour intervals, generally in the evening, for three days. An analysis of variance (AOV) was then run on each set of data to assist in the interpretation of results. The form used in the analysis of variance is given in figure 3.

Source	d.f.	S.S.	M.S.	F.
Total				
Mean				
Treatment				
Days				
Trt, x Days				
Error				

Fig. 3. The analysis of variance form used when preparing the raw data for interpretation.

RESULTS AND DISCUSSION

EXPERIMENTAL UNIT. It was found that with the consumption and check tubes located inside a rectangular container, tubes in certain positions had more liquid consumed from them than those in other positions. The further a tube was located from a corner, the lower the consumption rate. A circular arrangement of tubes solved this problem and allowed for placement of more tubes.

In choosing the size tubing to be used, it was necessary to keep in mind that tubing too large would allow liquid to run out, and tubing too small would not allow air bubbles, formed at the lower end by evaporation or consumption, to rise. Each rising air bubble accounted for a drop in liquid level of approximately 3.5 mm.

When drilling the tube holes in the lids, it was desirable to make them of such diameter that the tubes would slide into position easily, but remain in a rigid perpendicular position. However, due to properties of the plywood lids, satisfactory holes could not be drilled. The tubes were aligned before the start of a test, but were knocked out of line by the weight and movement of the cockroaches on them. It was therefore necessary to construct the supporting wire frames described earlier.

It was found that approximately 35 adult cockroaches per unit gave the best results. Responses were more effectively observed with a larger number, however, the number was limited since cannibalism resulted when large numbers were confined in small areas.

PRELIMINARY TEST UNIT CHECKS. The validity of the test unit had to be established before any test data could be recognized as reliable. The evaporation rate was checked from position to position around the circle and from one circle to the other since the recorded evaporation rates of the inner circle were used to calculate consumption rates in the outer circle. An analysis of variance indicated there was no significant difference in evaporation rates at the 20% level of probability. The consumption rates were checked around the outer circle and there was no significant difference in consumption rates at the 10% level of probability.

When the rates of consumption for the solvents acetone and ethyl alcohol were checked, no significant difference in the consumption rates of acetone concentrations was demonstrated. However, ethyl alcohol was found to be attractant at 0.01% concentration and repellent at 20% concentration. It was necessary to consider this last concentration because of certain chemical preparations. A chemical that was received as a 5% solution in alcohol was diluted to make a series of concentrations starting at 1% and decreasing in concentration. As a result of this dilution the alcohol solvent then becomes a 20% solution holding the 1% concentration of chemical. The mean consumption rates and results of Duncan's "new multiple-range test" for the acetone and alcohol tests are given in the appendix, tables 3, 11, and 12.

CHEMICAL PREPARATION. Many chemicals were received for testing, however, some were eliminated because of insolubility in water, the standard test solvent. Some were received with sediment in the bottom of the bottles and these chemicals were tested by using only the supernatant liquid in preparation of chemical concentrations. Further attempts were made to evaluate any of those which showed significant responses.

The decision later made to reduce the number of concentrations per chemical to be tested, was based on the conclusion that if a chemical was of any value as an attractant, three selected concentrations would have been sufficient to demonstrate its attractancy.

CHEMICAL TESTING. During the screening of approximately 45 chemicals, 5 were recognized with at least some attractant qualities. Tables 2-6 record the mean consumption results. Ten repellents were also recognized and these results are recorded in tables 4-10. Chemicals with a significantly higher consumption rate than that of water were considered to be attractant and those with a significantly lower rate than that of water were considered to be repellents.

Some difficulty was encountered when setting up the final series of tests with attractant chemicals. There was a sediment in chemical number 2227 when received and this could only be eliminated by making the solution slightly acid. This, however, was highly repellent to the cockroaches and, therefore unsatisfactory. This chemical had to be tested at concentrations of 0.01% or lower. Because three of the chemicals demonstrated their attractiveness at the highest concentrations previously tested (0.1%), it was desirable to include the next higher concentration (1%) in the final test. However, two of the chemicals precipitated in water at that concentration forcing an alternate method.

The five attractant chemicals, each in two concentrations, were tested against each other using water as the check. Only one, 1-Glutamic acid (2227), and in both concentrations tested, demonstrated significantly higher consumption rates than that of water. The results are recorded in table 13.

EXPERIMENTAL LIMITATIONS. There were certain limitations involved in this work, perhaps the most important being the use of only those chemicals that could be diluted in water to the desired range of concentrations. Suspensions were also to be avoided, because of the sediment at the feeding opening. Also the design offered no means of distinguishing between mode of action of stimuli resulting in consumption or in rejection, however, some comments are in order. Stimuli causing rejection or repellency may be distinguished from those causing consumption, but the individual types of stimulants causing reaction within each of these two contrasts are not easily separated. In the small confining test units, definite separation of stimuli that may be attractants, releasers, or "arrestants" would not be likely. According to definition, the reactions to releasers and arrestants could be the same although the modes of action are different. When a cockroach was observed to walk around beneath the tubes, evidently sensing the vapors emitted, a resulting reaction might likely be classified as repellent, attractant, or arrestant, but probably not releaser; If the cockroach continued to consume 3 mm of liquid after testing, this stimuli might be classified as a releaser. Chemical orientation would be more difficult for the cockroaches, because of the mixture of vapors and limited air movement.

TEST INSECTS. The test insects were all subjected to the same climatic conditions and all received the same, more-than-sufficient, supply of food. There was some variation in the rearing containers used. Usually the cockroaches lost an antennae or leg and were replaced before they had grown old. The variation from these conditions was kept to a minimum.

Assuming that some of the chemicals may have had a slightly toxic

affect on the insects, although none appeared to be killed throughout the testing, at least three days, and usually more, were allowed for a recuperation period between each test in any particular test unit.

CONCENTRATION EFFECT. In some cases consumption rates were observed to be highest on the first day of testing indicating there was a decline in response as the tests progressed. However, since all chemicals which caused a significant attraction reaction the first day, caused a significant attraction reaction for the three-day test period (recorded in appendix tables), there seemed to be little need for concern over the matter. This statement is also confirmed by the fact that four of the five chemicals found to be attractant, were so at higher concentrations.

SUMMARY AND CONCLUSIONS

An experimental procedure was designed whereby *P. americana* could be given access to a variety of liquid chemicals and concentrations and their reactions observed. An objective was to screen chemicals in search for those with attractant qualities. Vertically suspended glass tubes closed at the top, held the test liquids. Two or three chemicals in three or five concentrations each were tested at one time. The data were collected by measuring in millimeters the liquid level change in each glass tube. From this was then subtracted an evaporation rate to yield a consumption rate. These data were then subjected to an analysis of variance, "F" test, and Duncan's new multiple range test for interpretation. The 5% level of probability was used for all the multiple range test calculations. During the screening tests five chemicals were found with consumption rates significantly higher than that of the water check, and were thus considered to be attractant. These chemicals, with their attractant concentrations are: 5-(4-Pyridyl-N-oxide)isothiuram chloride at 0.00001% (2217), 1-Glutamic acid at 0.1% (2227), 2-Acetamidopyridine at 0.1% (3369), 2-Amino-4chloro-6-methylpyrimide at 0.1% (2269), and ethyl alcohol at 0.01%.

Those chemicals which produced a repellent reaction, having a consumption rate significantly lower than that of water, are listed in table 1. In order to compare the five attractant chemicals, a test was set up with water as the check. Each chemical was represented in two concentrations. The mean consumption rates are given in table 13. Chemical number

2227, 1-Glutamic acid, was attractant in both concentrations tested and demonstrated a much higher consumption rate than all the others. Because of the nature of the chemical and limitations of the experimental design, it was impossible to test concentrations higher than the 0.1% found attractant. A one percent concentration formed needle like crystals which settled to the lower ends of the tubes. Wafer tests described by Price (1963) might be a more accurate testing procedure.

REFERENCES CITED

- Dethier, V. G., 1947. "Chemical insect attractants and repellents," Philadelphia, Blakiston, 289pp.
- Dethier, V. G., and L. E. Chadwich, 1948. Chemoreception in insects. *Physiological Reviews*. 28(2): 220-254.
- Dethier, V. G., 1957a. Chemoreception and the behavior of insects. *Survey of Biological Processes*. 3: 149-183.
- Dethier, V. G., 1957b. Insect attractants and repellents. *Soap and Chemical Specialties*. 33(3): 97.
- Dethier, V. G., L. Barton Browne, and Carroll N. Smith, 1960. The designation of chemicals in terms of the responses they elicit from insects. *Journal Economic Entomology*. 53(1): 135.
- Hermes, W. B., and Y. Nelson, 1913. The croton bug (*Ectobia germanica*) as a factor in bacterial dissemination, *American Journal Public Health*, 3: 929-934.
- Hodgson, E. S., 1958. Chemoreception in arthropods. *Annual Review of Entomology*. 3: 19-36.
- Jacobson, M., and M. Berzoza, 1963. Chemical insect attractants. *Science*. 140(3574): 1367-1373.
- Price, Richard G., 1963. The evaluation of repellents with four species of roaches. Ph. D. Thesis. Oklahoma State Univ., Stillwater, Oklahoma.
- Roys, C. C., 1954. Olfactory nerve potentials a direct measure of chemoreception in insects. *Annual New York Academy of Science*. 58(2): 250-255.
- Roys, C. C., 1956. A comparison between the thresholds of taste receptors and that of non-gustatory nerve tissue to taste stimuli in the cockroach. *Anatomical Record*. 125: 555-556.
- Sun, Yum-Pel., 1960. Pre-test conditions which affect insect reaction to insecticides. In Shepard, H. H., ed. *Methods of testing chemicals on insects*. 2: 1-9. Minneapolis, Burgess.

Wharton, D. R. S., G. L. Miller, and M. L. Wharton, 1954a. The odorous attractant of the american cockroach Periplaneta americana (L.). I. Quantitative aspects of the response to the attractant. Journal of General Physiology. 37(4): 461-469.

Wharton, D. R. A., G. L. Miller, and M. L. Wharton, 1954b. The odorous attractant of the american cockroach Periplaneta americana (L.). II. A bioassay method of the attractant. Journal of General Physiology 37(4): 471-481.

APPENDIX

APPENDIX

Table 2. Mean consumption of chemicals 2217 and 3329 at several concentrations.^a

Chemical Number	Chemical Concentration in percent	Mean Consumption in millimeters
2217	0.1	-0.167
3329	0.0001	0.83
3329	0.1	1.0
2217	0.001	2.0
3329	0.001	2.33
2217	0.0001	2.67
water check		2.75
2217	0.01	3.0
3329	0.00001	3.0
3329	0.01	4.0
2217	0.00001	10.5

^aThere was a significant difference between any two means not connected by the same line. Result of six observations, mean square-19.461.

^bA negative number resulted when the level drop in the evaporation check tube was more than that in the consumption tube.

Table 3. Mean consumption of ethyl alcohol at several concentrations.^a

Chemical Concentration in percent	Mean Consumption in millimeters
0.1	-0.166
0.0001	0.417
water check	1.17
0.001	2.33
0.00001	2.92
0.01	6.08

^aTwelve observations, mean square-15.236.

Table 4. Mean consumption of chemicals 2269, 3381, and 2271 at several concentrations.^a

Chemical Number	Chemical Concentration in percent	Mean Consumption in millimeters
3381	0.001	-3.5
2271	0.1	0.167
water check	--	1.19
2269	0.00001	2.17
2269	0.001	2.5
2271	0.00001	2.67
3381	0.00001	3.17
2271	0.001	3.17
3381	0.1	4.0
2269	0.1	6.67

^aSix observations, mean square=8,392

Table 5. Mean consumption of chemicals 3367, 3369, and 2259 at several concentrations.^a

Chemical Number	Chemical Concentration in percent	Mean Consumption in millimeters
2259	0.1	-1.83
3367	0.1	0.166
2259	0.00001	0.166
3369	0.00001	2.17
3367	0.00001	2.5
3369	0.001	2.67
3369	0.001	3.33
2259	0.001	3.33
water check	--	6.7
3369	0.1	19.17

^aSix observations, mean square=15.142.

Table 6. Mean consumption of chemicals 2321, 2227, and 3343 at several concentrations.^a

Chemical Number	Chemical Concentration in percent	Mean Consumption in millimeters
3343	0.1	-1.67
3343	0.001	0.5
2321	0.1	0.667
3343	0.00001	2.33
2227	0.00001	2.83
2321	0.00001	3.0
2321	0.001	3.34
2227	0.001	5.5
water check	--	6.17
2227	0.1	13.34

^aSix observations, mean square=25.306.

Table 7. Mean consumption of chemicals 3225 and 2117 at several concentrations.^a

Chemical Number	Chemical Concentration in percent	Mean Consumption in millimeters
2117	0.1	-1.17
2117	0.01	2.17
2117	0.0001	3.67
3225	0.0001	3.67
2117	0.001	4.0
2117	0.00001	4.33
3225	0.00001	4.67
water check	--	5.25
3225	0.001	5.5
3225	0.01	6.67

^aSix observations, mean square=12.378.

Table 8. Mean consumption of chemicals 2229, 3351, and 3353 at several concentrations.^a

Chemical Number	Chemical Concentration in percent	Mean Consumption in millimeters
2229	0.1	-0.83
3351	0.00001	0.167
3351	0.1	1.33
3353	0.1	2.0
3353	0.001	3.17
2229	0.001	3.17
3351	0.001	4.33
3353	0.00001	7.17
2229	0.00001	7.83
water check	--	9.73

^aSix observations, mean square-11.627.

Table 9. Mean consumption of chemicals 3363, 2253, and 3365 at several concentrations.^a

Chemical Number	Chemical Concentration in percent	Mean Consumption in millimeters
3365	0.1	-1.5
3363	0.1	0.17
3363	0.1	0.33
3363	0.01	0.5
2253	0.00001	0.5
3365	0.001	2.67
2253	0.001	2.83
water check	--	4.0
3363	0.00001	4.83
3365	0.00001	6.83

^aSix observations, mean square-12.166

Table 10. Mean consumption of chemicals 3371, 2261, and 3373 at several concentrations,^a

Chemical Number	Chemical Concentration in percent	Mean Consumption in millimeters
3373	0.1	-1.7
3371	0.1	0.17
2261	0.1	0.17
3371	0.001	0.883
2261	0.00001	2.67
2261	0.001	3.67
3373	0.001	4.33
water check	--	4.4
3373	0.00001	6.83
3371	0.00001	7.0

^aSix observations, mean square=22.623.

Table 11. Mean consumption of acetone at several concentrations.^a

Chemical Concentration in percent	Mean Consumption in millimeters
20.0	0.75
0.002	1.08
0.2	2.25
2.0	2.33
water check	3.0
0.02	4.50

^aTwelve observations, mean square=12.305

Table 12. Mean consumption of ethyl alcohol at several concentrations.^a

Chemical Concentration in percent	Mean Consumption in millimeters
20.0	-0.67
2.0	1.08
0.02	2.0
water check	3.5
0.2	3.75
0.002	4.0

^aTwelve observations, mean square-10.546

Table 13. Mean consumption of the chemicals found to be attractant to *Periplaneta americana*.^a

Chemical Number	Chemical Concentration	Mean Consumption in millimeters
3369	1.0	-0.58
2269	0.1	-0.33
2217	0.00001	1.25
alcohol	0.01	1.5
2269	0.01	1.67
2271	0.000001	2.75
water check	--	2.83
3369	0.1	3.0
alcohol	0.001	3.08
2227 ^b	0.01	14.75
2227	0.1	18.42

^aTwelve observations represented, mean square-22.288.

^bThere was a slight suspension present in the chemical while testing.

VITA

Donald Matthew McCroddan

Candidate for the Degree of

Master of Science

Thesis: SENSORY DISCRIMINATION OF CHEMICALS BY Periplaneta americana
(L.).

Major Field: Entomology

Biographical:

Personal Data: Born in Glenridge, New Jersey, August 23, 1938,
the son of Lincoln and Laura McCroddan.

Education: Graduated from Bloomfield High School, Bloomfield,
New Jersey; received the Bachelor of Arts degree from the
John Brown University, with a major in Biology, in May,
1961; completed requirements for the Master of Science
degree in August, 1963.

Experience: Cotton scouting, summer of 1961; research assistant,
Oklahoma State University, summers of 1962-63.

Organizations: Phi Sigma, Sanborn Entomology Club.