Bionomic investigations of Aedes nigromaculis (Ludlow) (Diptera: Culicidae) with special reference to oviposition
by Jack W Warren

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Entomology
Montana State University


Abstract:
On-field production of irrigation-water mosquito larvae in the Milk River Valley, Montana is discussed. Certain topographical features of this area that favor oviposition of these mosquitoes are noted. Aedes nigromaculis is chosen as a representative species for study. Field investigations show that oviposition under field conditions is not at random, but is favored by locations on the sides of small depressions and thick layers of organic debris on the ground surface. A study of larval hatch and development indicates that larval movement away from hatching sites occurs, and that about a week is necessary to complete larval and pupal development.

Olfactometer tests show that certain organic decomposition gases such as methane, carbon dioxide, and hydrogen sulfide are apparently not oviposition attractants. Individual mosquitoes isolated in oviposition and substance choice vials yield data on egg production, oviposition expectation, and the ability of ovipositing females to detect chemical differences in their substrata. These data indicate that a chemotactic sense exists in these mosquitoes. However no evidence was found to show that chemical differences, such as alkalinity variations, likely to be found in their natural habitat has any effect on their oviposition site choice. Evidence from analysis of air samples collected in the field indicates that measurable amounts of carbon dioxide do evolve from the soil in irrigated pastures.

Field observations show that pupae can survive and develop into adults in the absence of water.

# BIONOMIC INVESTIGATIONS OF *AEDES NIGROMACULIS* (LUDLOW) (DIPTERA:CULICIDAE) WITH SPECIAL REFERENCE TO OVIPOSITION

by

JACK W. WARREN

A THESIS

Submitted to the Graduate Faculty

in

partial fulfillment of the requirements

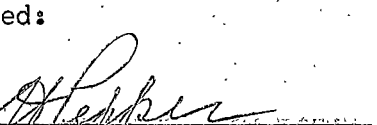
for the degree of

Doctor of Philosophy in Entomology

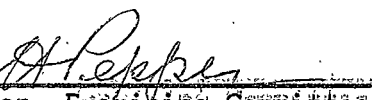
at

Montana State College

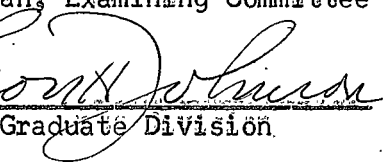
Approved:



Head, Major Department



Chairman, Examining Committee



Dean, Graduate Division

Bozeman, Montana
September, 1958

TABLE OF CONTENTS

ABSTRACT

On-field production of irrigation-water mosquito larvae in the Milk River Valley, Montana is discussed. Certain topographical features of this area that favor oviposition of these mosquitoes are noted. Aedes nigromaculis is chosen as a representative species for study. Field investigations show that oviposition under field conditions is not at random, but is favored by locations on the sides of small depressions and thick layers of organic debris on the ground surface. A study of larval hatch and development indicates that larval movement away from hatching sites occurs, and that about a week is necessary to complete larval and pupal development.

Olfactometer tests show that certain organic decomposition gases such as methane, carbon dioxide, and hydrogen sulfide are apparently not oviposition attractants. Individual mosquitoes isolated in oviposition and substance choice vials yield data on egg production, oviposition expectation, and the ability of ovipositing females to detect chemical differences in their substrata. These data indicate that a chemotactic sense exists in these mosquitoes. However no evidence was found to show that chemical differences, such as alkalinity variations, likely to be found in their natural habitat has any effect on their oviposition site choice. Evidence from analysis of air samples collected in the field indicates that measurable amounts of carbon dioxide do evolve from the soil in irrigated pastures.

Field observations show that pupae can survive and develop into adults in the absence of water.

## INTRODUCTION

Prolific mosquito production on irrigated pasture lands has long been a problem in the western United States. The Milk River project in northern Montana is such a problem area.

In order to better understand the mosquito problem in this area, a cooperative program of research was initiated by the Montana Agricultural Experiment Station, the Montana State Board of Health, the Communicable Disease Center of the U. S. Public Health Service and the Soil and Water Conservation Research Branch of the Agricultural Research Service of the U. S. Department of Agriculture. The present research was undertaken as one phase of this program. Its basic aim was to gain a better understanding of some of the factors influencing mosquito oviposition behavior in irrigated pastures. The problem was approached in the following ways:

1. By pinpointing on-field oviposition sites by taking sod samples from various locations in an irrigated pasture known to produce great numbers of mosquitoes. This was to see if the females were exhibiting a definite preference for oviposition sites with respect to micro-ecological differences found within the field (i.e. irregularities of surface, plant densities, ground cover, moisture etc.), or were merely scattering their eggs indiscriminately over the surface of the ground.

2. By obtaining information about hatching time, amount of larval movement away from hatching sites, and larval development time.

3. By obtaining information about the time necessary after a blood meal to "mature" eggs, the number of eggs a female mosquito can be expected

to lay, etc.

4. By testing the ability of gravid female mosquitoes, (when given a choice) to distinguish between various naturally occurring gases, certain solutions, and substrate differences ("texture", color, etc.) as possible oviposition stimulants.

Aedes nigromaculis was chosen as the experimental species because of its abundance in the Milk River Valley throughout the summer, and because it is easily separated by macroscopic characteristics from the several other common species in this area.

There are many studies of certain phases of oviposition behavior reported in the literature. Gjullin et al. (1950) working with A. vexans and A. sticticus in Washington and Oregon, and Bodman and Gannon (1950) in Illinois studies of A. vexans, found that oviposition was not random, and tended to be concentrated along the sides of depressions.

Many workers, among them Mayne (1926), Thomson (1938), Kennedy (1942), Parker (1948), Parker (1952) and Wallis (1955) have noted that other species of mosquitoes can utilize differences in amounts of water vapor in the air, in temperatures, and in free water locations to orient themselves to oviposition sites. Wallis (1954 B), Beckel (1955) and Trembley (1955) have reported that certain Aedes species are able to distinguish between different colors of substrate, differences in "texture" of substrate, and differences in light-and-dark areas and that they make oviposition site choices on the bases of these abilities.

Various workers have reported on the ability of mosquitoes to utilize chemical stimuli, by way of distinguishing between different chemicals in solutions and as gases, to make oviposition site choices. Noteworthy among these are the studies of Sharma and Sen (1921), Rudolfs (1922), Crumb (1924), Wallis (1954 A), and Wallis (1954 C). Dethier (1947) devoted an entire book to summarizing the many excellent papers available on the chemical senses of insects.

Certain gases, among them carbon dioxide, methane, and hydrogen sulfide, are produced by bacteria decomposing organic material. There are thick layers of these decaying materials present in areas chosen by irrigated pasture mosquitoes for egg laying sites. Therefore it was one of the primary aims of the present research to investigate the possibility that the above mentioned gases might be oviposition stimuli. Crumb (1924) found evidence that methane may be an oviposition stimulus for Culex pipiens. That mosquitoes are attracted by carbon dioxide as a feeding stimulus has been demonstrated by many workers, such as Rudolfs (1922), Brown et al. (1951), Reeves (1951), Willis and Roth (1952), Reeves (1953) and Laarman (1955). In the present research, it was thought possible that in lesser concentration carbon dioxide might be an oviposition attractant as well.

ACKNOWLEDGEMENTS

For their aid, encouragement and helpful criticism I wish to thank the following personnel of Montana State College; Mr. E. B. Hastings,

Mr. J. H. Pepper, and Mr. R. C. Froeschner. Thanks for aid are also due to other members of the Zoology and Entomology Department, Chemistry Department, and Chemical Engineering Department of Montana State College, and to U. S. Public Health and Agricultural Research Service personnel stationed at Chinook, Montana. Grateful acknowledgement is given to the National Institutes of Health for providing the funds for this research, and to my wife, Ellen, for her encouragement and help in preparing the manuscript.

## DESCRIPTION OF THE STUDY AREA

The Milk River Valley lies in a glaciated section of the Missouri plateau in north central Montana. The elevation of the area varies from 2068 to 2445 feet above sea level and is characterized by a gently rolling prairie dissected by the relatively narrow river valley which is approximately 100 feet lower than the bordering plain. The valley floor appears flat upon gross examination, but upon closer inspection is uneven in many places. These surface irregularities together with lack of slope (only about two feet per mile), and high clay content of the soil (over 80 percent clay in some areas) complicate irrigation and surface drainage. Irrigated native grass pastures are generally not level and water tends to collect in low spots (see Fig. 1.). Here, due to the fact that these clay soils "seal" quickly and do not allow much water infiltration, the water remains until it evaporates. Irrigation systems in these fields are generally of the "contour ditch" type (see Fig. 2). This further complicates

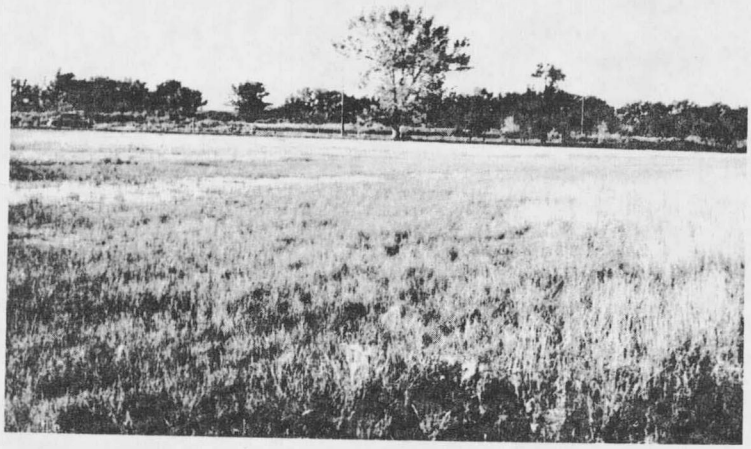


Fig. 1. Typical western wheatgrass pasture showing residual surface water ideal for mosquito production.

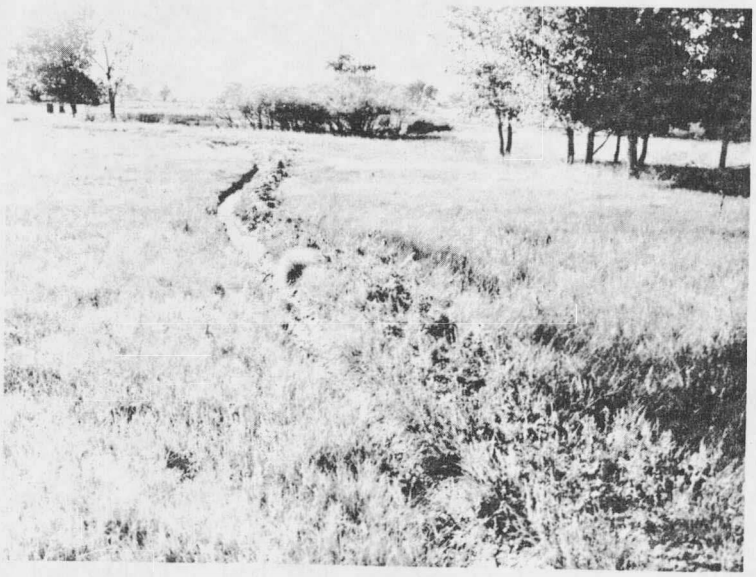


Fig. 2. A contour ditch irrigation system.

the problem of standing water, since these distribution ditches often contain water even after all water has disappeared from the fields themselves.

In a report on a study of mosquito breeding conditions in this area (Anonymous-1953), U. S. Public Health Service personnel indicated that irrigated pastures produced about 43 percent of the total on-field mosquito production found. Furthermore, according to their data, surface pools were responsible for 99 percent of the total breeding on native grass pastures.

## 1955 SOD EGG HATCH EXPERIMENTS

### Methods

For the determination of natural oviposition sites, circular sod samples 5 1/2 inches in diameter (23.72 square inches) and one inch deep were cut from various areas in a western wheatgrass (Agropyron smithii) pasture known to produce very high populations of mosquitoes.

These samples were taken at locations across the slopes of small depressions and from relatively level areas within the field. A record was kept of the exact place where each sample was taken, and plant counts and duff layer estimates also were made on each sample. After excess grass was clipped to facilitate observation, the samples were placed in half gallon battery jars for flooding. The jars were flooded to a depth of three inches above the sample surface, using water from the irrigation ditch serving the field. The water was strained through standard milk filter discs to preclude introduction of any eggs or larvae.

The term "hatch" refers to the appearance of one or more larva(e)

from a sample. Nine sets of 24 samples each were taken over a three week period beginning June 1, 1955. Each set was taken from a different locality in the field. Two of the nine sets were taken entirely from level areas, and the remaining sets consisted of transect samples across depressions from side to side. All samples (24) in each set were taken the same day, and the processing started on these before another set was taken.

Frequent inspections were made to detect hatching, most of which took place within 12 hours, and was usually complete by about 24 hours. Forty-eight hours after flooding, the larvae were removed with a pipette for counting. Attempts were made to rear these larvae to the fourth instar for identification but mortality was excessive due to scum formation or from some other cause, possibly a fungus infection. The few larvae that were reared through were mostly A. nigromaculis, with some A. dorsalis.

The samples were kept submerged for five days to allow for any delayed hatching. Those which showed no hatch in the five days were discarded, and those showing a hatch were air dried for a week and then flooded again to check for a rehatch. One sample crumbled so badly it had to be discarded for rehatch.

In these studies any hatch in the first flooding of the samples was used as an indication of these habitats being chosen as egg laying sites. The possibility exists that repeated flooding would have shown the presence of eggs even in those samples which showed no hatch on the first flooding. It was felt that the number of larvae which hatched would not be any better

criterion of oviposition preference unless complete hatch could be effected, which was impossible with the facilities and time available.

## Results

Table I. Shows the results obtained from the hatch experiments. It will be noted from Table I. that three sets of 24 samples each (sets 3, 7, and 9) showed no larval hatch. One of these sets was from a level area, and two were from areas containing depressions. This would indicate that some areas in a field may be more favorable for oviposition than other areas which appear similar (in physical aspects) to the human eye. The data also show that there is an apparent preference for the sides of depressions as oviposition sites. These results are comparable to the results of Bodman and Gannon (1950) working with A. vexans in Illinois.

Table I. Egg deposition in relation to slope of terrain as indicated by hatch experiments.

| | Depression Sides | | Depression Bottoms | | Level Areas | |
|---|---|---|---|---|---|---|
| Set No. | No. of Samples Taken | No. of Samples W/Hatch | No. of Samples Taken | No. of Samples W/Hatch | No. of Samples Taken | No. of Samples W/Hatch |
| 1 | 17 | 4 | 7 | - | - | - |
| 2 | 15 | 5 | 5 | 1 | 4 | - |
| 3 | 8 | - | 5 | - | 11 | - |
| 4 | 10 | 5 | 4 | - | 10 | 5 |
| 5 | - | - | - | - | 24 | 5 |
| 6 | 13 | 7 | 9 | 2 | 2 | - |
| 7 | - | - | - | - | 24 | - |
| 8 | 11 | 4 | 2 | - | 11 | 1 |
| 9 | 9 | - | 6 | - | 9 | - |
| Totals | 83 | 25 | 38 | 3 | 95 | 11 |
| | | Depression Sides | | Depression Bottoms | | Level Areas |
| Percent of Samples Showing Hatch | | 30 | | 8 | | 12 |

The number of larvae obtained from the first flooding of the 39 samples of sod which showed a hatch varied from 1 to 63 per sample. Thirty-eight of the samples had 39 or fewer larvae hatch per sample. Twenty-two samples produced a hatch on second flooding, and in 11 of these the hatch was larger in the second flooding than in the first.

Table II. shows the relationship of amount of organic debris or "duff" on the surface of the ground and oviposition preference as evidenced by hatch obtained.

From the data presented in Table II. it would appear that the quantity of duff cover may have either a direct or indirect effect on the selection of oviposition sites by the mosquito species involved, with preference shown for thick layers of duff. No explanation can be given for the fact that three light duff samples out of one set showed larval hatch.

Analysis of the data obtained from counting plant numbers on the samples show no correlation with oviposition preference, as can be seen by looking at Table III. The plant complex in these samples consisted largely of western wheat grass, several species of sedges, and annual weeds. Observations made during these studies indicate that a measure of density of plant cover might have shown a more direct relationship. This is because one large clump of grass (classified as one plant) may give several times as much ground cover as four or five small annual weed plants or grass sprouts.

Table II. Results of oviposition studies in relation to quantity of duff cover per sample.

| Set No. | Very Heavy Duff Cover* | | Heavy Duff Cover | | Moderate Duff Cover | | Light Duff Cover | |
|---|---|---|---|---|---|---|---|---|
| | No. of Samples Taken | No. of Samples W/Hatch | No. of Samples Taken | No. of Samples W/Hatch | No. of Samples Taken | No. of Samples W/Hatch | No. of Samples Taken | No. of Samples W/Hatch |
| 1 | 2 | 1 | 10 | 2 | 11 | 1 | 1 | - |
| 2 | 1 | 1 | 9 | 2 | 8 | - | 6 | 3 |
| 3 | - | - | 14 | - | 10 | - | - | - |
| 4 | 1 | 1 | 20 | 8 | 3 | 1 | - | - |
| 5 | 2 | 2 | 16 | 3 | 6 | - | - | - |
| 6 | 7 | 4 | 14 | 5 | 3 | - | - | - |
| 7 | - | - | 12 | - | 10 | - | 2 | - |
| 8 | - | - | 13 | 3 | 10 | 2 | 1 | - |
| 9 | 1 | - | 8 | - | 14 | - | 1 | - |
| Totals | 14 | 9 | 116 | 23 | 75 | 4 | 11 | 3 |

| | Very Heavy Duff Cover | Heavy Duff Cover | Moderate Duff Cover | Light Duff Cover |
|---|---|---|---|---|
| Percent showing Hatch | 64 | 20 | 5 | 27 |

* Very Heavy Duff Cover - Refers to a dense layer of plant debris 1/2 inch or more deep and entirely covering the sample's surface.

Heavy Duff Cover - A layer of plant debris entirely covering the sample's surface.

Moderate Duff Cover - Considerable plant debris, but with some spots only thinly covered or bare.

Light Duff Cover - Only small amounts of plant debris, most of sample thinly covered or bare.

Table III. Results of oviposition studies in relation to number of plants per sample.

| Set No. | No. Plants per Sample: 1-9 No. of Samples Taken | 1-9 No. of Samples W/Hatch | 10-19 No. of Samples Taken | 10-19 No. of Samples W/Hatch | 20-29 No. of Samples Taken | 20-29 No. of Samples W/Hatch | 30 or More No. of Samples Taken | 30 or More No. of Samples W/Hatch |
|---|---|---|---|---|---|---|---|---|
| 1 | - | - | 6 | 1 | 18 | 3 | - | - |
| 2 | 8 | 2 | 12 | 3 | 4 | 1 | - | - |
| 3 | - | - | 5 | - | 14 | - | 5 | - |
| 4 | - | - | 19 | 6 | 4 | 3 | 1 | 1 |
| 5 | - | - | 6 | - | 16 | 4 | 2 | 1 |
| 6 | - | - | - | - | 4 | 1 | 20 | 8 |
| 7 | - | - | 8 | - | 15 | - | 1 | - |
| 8 | - | - | 4 | 1 | 13 | 4 | 7 | - |
| 9 | - | - | 15 | - | 8 | - | 1 | - |
| Totals | 8 | 2 | 75 | 11 | 96 | 16 | 37 | 10 |

| | 1 - 9 Plants | 10-19 Plants | 20-29 Plants | 30 or More |
|---|---|---|---|---|
| Percent showing hatch | 25 | 15 | 17 | 27 |

## RING STUDIES FOR LARVAL DEVELOPMENT

### Methods

Twenty-four isolation rings, 1 1/2 feet in diameter and 12 inches deep were constructed from galvanized sheet metal. Each was fitted with a removable screen cover. These rings were similar to those used by Edmunds (1955) to isolate egg rafts of Culex tarsalis.

At various locations in the same field where the sod samples were taken, these rings were driven into the soil to a depth of six inches and sealed around the edges with mud. Water was then poured in until it stood about four inches deep. It was planned to make observations on the time for egg hatch after flooding, and the time for larval development. These rings were likewise to be used to determine the number of larvae produced in a particular habitat.

### Results

A great deal of difficulty was experienced with the use of these rings. First, it was difficult to maintain water in the rings in some locations due to seepage. Secondly, temperatures of the water were much cooler than those of field water in the same vicinity if the screen covers (which provided shade) were left on. If the covers were removed, it was impossible to prevent a very rapid influx of predators, principally Hemiptera (Notonectidae and Corixidae) and Coleoptera (Dytiscidae and Hydrophilidae). These caused a very high mortality in a short time. Also, the water in the rings became stagnant very rapidly and a scum formed which was lethal to the larvae. In view of these difficulties

use of these rings was discontinued.

## LARVAL DEVELOPMENT FIELD STUDIES

### Methods

These studies were started to determine to what extent larvae were washed from place to place in a field by the irrigation water, but was later continued to follow through the time necessary for development from egg to adult.

A study plot with an area of 35,250 square feet was constructed in an irrigated pasture by throwing up dikes of earth to prevent water run-off. Dominant vegetation on this area was western wheatgrass. Water was started on the plot at 10:30 a.m., August 9, 1955 and was continued for 31 hours. At the end of this period the area was flooded to an average depth of approximately four inches (maximum about six inches, minimum about two inches). This amount and method of water application is similar to that used by the farmers in the area.

The larval population was sampled by the use of a white enamel dipper, as described by Carpenter and LaCasse (1955), Mail (1934) and others. The plot was sampled at 15 minute intervals the first four hours from the start of flooding to determine the time lapse before larval hatch began. To check the possibility of larvae being washed into the plot in the irrigation water the ditch was sampled periodically. No larvae were found in 200 dips taken in the ditch water, nor were any seen.

After the hatching was nearly complete (24 hours), the plot was checked twice daily, morning and afternoon, for the first seven days. One observation a day was taken after this time. Water and air temperatures were recorded throughout the period with a recording thermograph. Daily air temperatures ranged from lows of 40-45° F. at about 5:30 a.m. to highs of 80-94° F. at about 2 p.m. Water temperatures followed a similar pattern with lows of 52-62° F. early in the mornings to highs of 76-90° F. in the middle of the afternoons. This daily pattern of temperature fluctuation was fairly consistent throughout the test.

For the sake of uniformity of sampling, the plot was divided into nine smaller areas each of which was dipped 25 times per sample. It was possible from these samples to determine rather closely the beginning of larval hatch and the transition of one instar to the next.

## Results

The pasture in which this study plot was located had not been flooded (except by spring thaw) for at least two years prior to this test. Consequently the first flooding produced only a few larvae. The water from this first flooding was therefore drained to provide a suitable habitat for egg deposition, and the larval production from the second flooding (10 days after the first) was used in this study.

The first larvae were seen about 3 1/4 hours after the water was started on the plot. This would indicate that embryonic development in A. nigromaculis, the species obtained in these studies, was similar to that

found in other species as reported by Gjullin, et al. (1950). The head capsules of A. nigromaculis larvae are white when first emerged, and gradually darken over a three to five hour period as pigmentation develops. Using this phenomenon as an indication, visual observations showed that larvae were washed along by the moving water, since older larvae of the first instar (with dark heads) and newly hatched larvae (with white heads) were observed together just behind the advancing edge of the water sheet. Figure 3. shows the instar development pattern as indicated by this study. It can be seen that the first three instars develop rapidly, each of the first three stadia requiring about three days for all of the larvae to pass through. The fourth stadium persisted for about six days, and the pupal stage for about five days. Some larvae developed much more rapidly than others, and most of the first adults to emerge were males.

Some of the first pupae found were collected and placed in a confining ring so that adult emergence could be checked. Judging from the cast pupal skins remaining in the ring, the first adults emerged sometime between 6:40 p.m., August 15, and 1:00 p.m., August 16. This was about 6 1/2 days after the plot was flooded. However, the main emergence of adults occurred between 2:15 p.m., August 17, and 10 a.m., August 18, or approximately eight days after flooding. At this latter time hundreds of newly emerged adults were observed clinging to the grass protruding from the water, and many were seen still in the act of emergence. Both males and females were seen, in approximately equal numbers. Swarms of these mosquitoes flew up upon disturbance, but immediately settled back to the grass. Very few of the

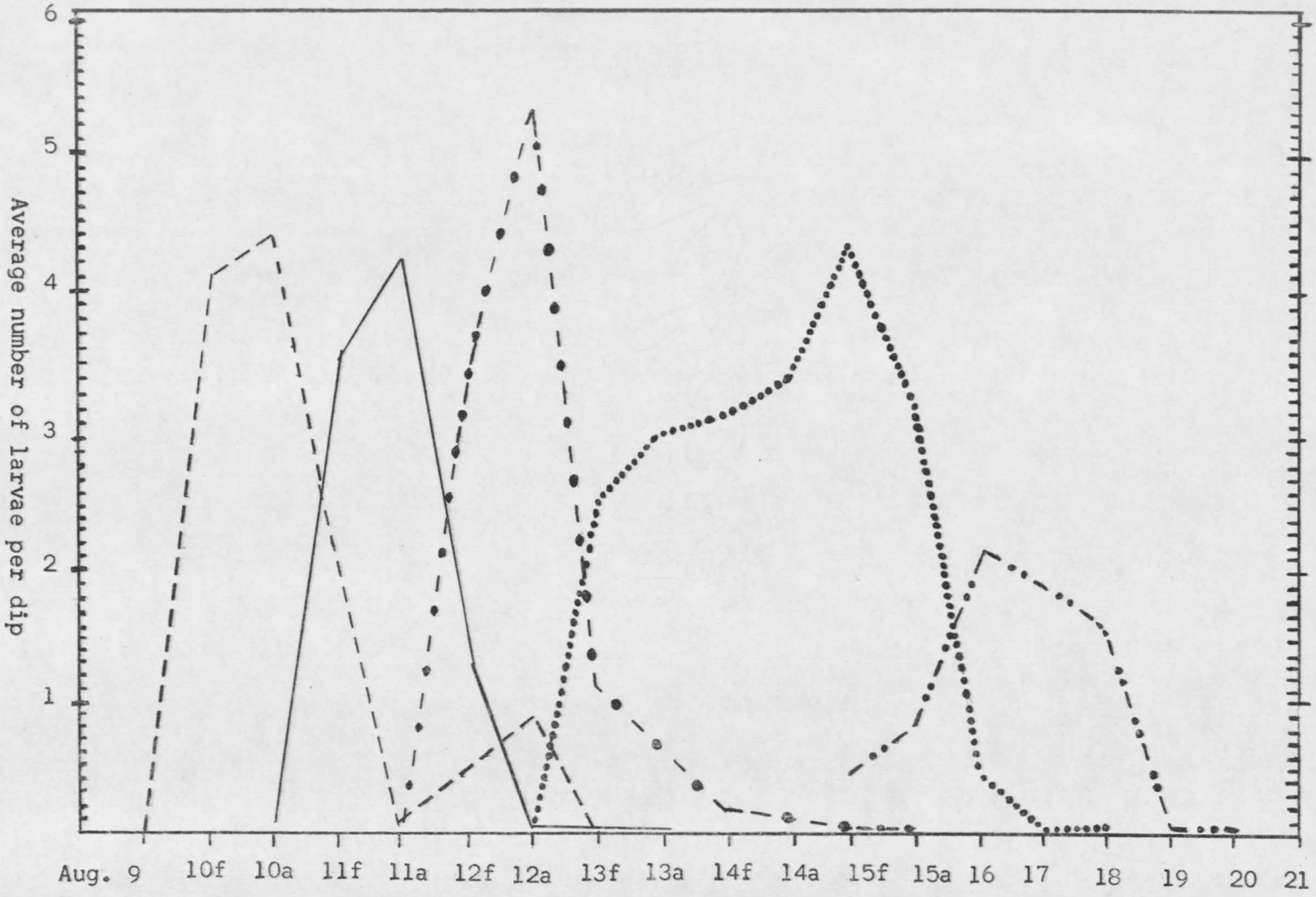



Fig. 3. Larval instar durations of A. nigromaculis.

females offered to bite even though the observer had applied no insect repellant. By 3:05 p.m., August 19, the time of the next observation, very few males were seen. The females were evidently ready for a blood meal at this time, for the observer was attacked by swarms of voracious females even after a heavy application of insect repellant to all exposed skin. By 10 a.m., August 20, or 11 days after flooding started, most of the mosquitoes had disappeared from the area, and comparatively few adults were seen.

Why the average number of third instar larvae captured per dip was higher (as is shown in Fig. 3.) than for the other instars was not readily apparent. Perhaps for some reason these larvae showed less avoidance to the dipper than did the other instars. The much lower peak for the pupae indicated in Fig. 3. was due, at least in part, to the fact that as the adults emerged the total number of pupae dropped correspondingly.

## OLFACTOMETER EXPERIMENTS

### Methods

In order to test the effects of methane and carbon dioxide as oviposition attractants for A. nigromaculis, an insect olfactometer was built during the spring of 1956. Its design was modified from those used by Brown et al. (1951), Willis (1947), Willis and Roth (1952), and Laarman (1955).

The olfactometer was constructed of 3/8 inch plywood lined on the inside with two layers of beaver board, held apart with wood spacers.

This arrangement provided two "dead" air spaces for insulation. The entire front of the olfactometer was removable for convenience of servicing, and was equipped with a glass observation window, and a netting "sleeve" opening to allow insertion of an arm to transfer mosquitoes. The preliminary design used in 1956 was a "chamber-within-a-chamber" plan (see Fig. 4.) so that the inner chamber which actually enclosed the mosquitoes was within an outer chamber designed to maintain constant temperatures. The outer chamber was equipped with a 3/4 horsepower commercial refrigeration unit and also thermostatically controlled heating elements so that the air could be heated or cooled as desired. A fan attached to the refrigerator coil in the outer chamber provided air circulation. The inner chamber was constructed of 3/16 inch masonite to allow good heat exchange, and had glass panels in the ceiling to allow light to enter.

Small screened openings in the walls of the inner chamber provided air exchange between the outer and inner chambers to allow equilibria of temperature and humidity to be reached without objectional strong air currents in the inner chamber. Lights for observation were provided on the ceiling of the outer chamber in the form of six 40 watt lumiline bulbs connected to a Powerstat rheostat so that light intensity could varied. Evaporation pans placed beneath the floor of the inner chamber provided the water vapor necessary to maintain relative humidity at a level favorable for the mosquitoes. By varying the number of pans, some control of relative humidity was obtained, but it was impossible to regulate it within close limits (subsequent modifications in 1957 allowed very much

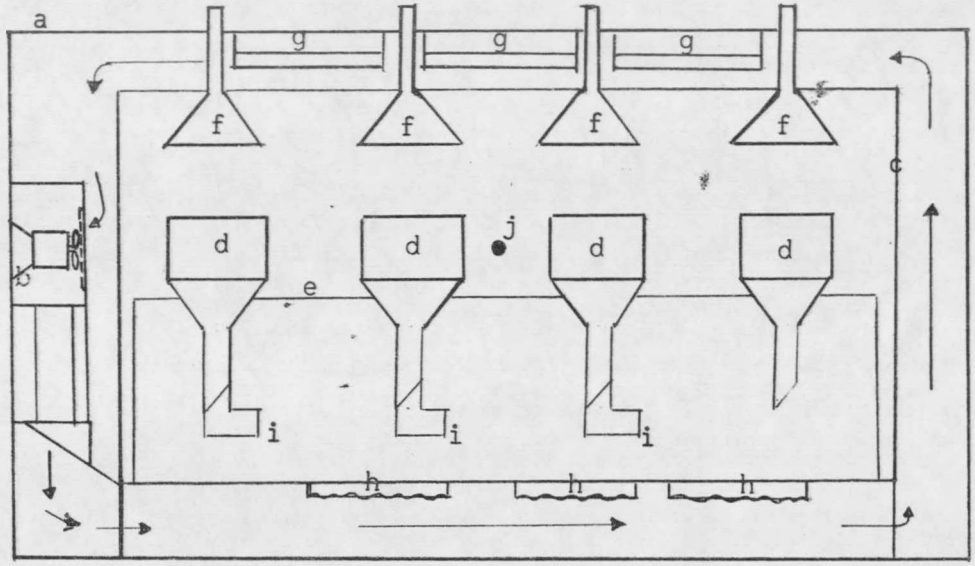



Fig. 4. 1956 experimental chamber - Diagrammatic-not to scale.

a. Outer chamber wall
b. Fan and refrigerator coil
c. Inner chamber wall
d. Buchner funnels
e. Funnel stand
f. Exhaust funnels
g. Lights
h. Heating elements
i. Plastic hoses leading to gas apparatus
j. Heater thermostat
→ Direction of air flow

Chamber Dimensions

Outer Chamber
Length - 60"
Height - 30"
Depth - 30"

Inner Chamber
Length - 41"
Height - 17"
Depth - 26"

improved humidity, as well as temperature, control).

Oviposition sites were provided by wet white turkish toweling fastened over the mouths of four seven-inch porcelain Buchner funnels held upright in the chamber by a wooden stand. These Buchner funnels were used so that the gas mixtures to be tested could be introduced through plastic tubing into the funnels and then be passed through the toweling. This arrangement allowed four choices to be available to the mosquitoes; in the gas experiment one funnel had a methane-air mixture passing through it, one a carbon dioxide-air mixture, one pure air, and the fourth with no gas-air or air flow. The toweling was kept saturated by means of a cloth wick in a small container of water inside each funnel. Thus the mosquitoes had no access to moisture other than the toweling. Four Petri dishes containing cellucotton pads saturated with 10 percent sucrose solution were placed in the chamber. These provided food for the mosquitoes as described by Trembley (1955).

Twelve experiments were conducted in this preliminary design olfactometer. The first 11 of these were carried out with the idea in mind of finding the effects of varying temperatures and humidities upon oviposition, and no air or gas mixes were passed through the funnels in these experiments. Preliminary trials with varying light intensities indicated that the heat produced by the bulbs caused too great a fluctuation in temperature, so all of the experiments were carried out in darkness. At the termination of the 55° F. test (test No. 9) 45 of the mosquitoes that still appeared to be in a "healthy" condition were removed from the test chamber

and were placed in a pint jar with a layer of wet cellucotton, covered by white filter paper, on the bottom. This jar was placed in the laboratory and allowed to reach room temperature (approximately 75° F.). This was done to see if these mosquitoes, which had laid no eggs in 114 hours at 55° F., would lay eggs when their environmental temperature increased to a more favorable level. For the twelfth experiment, involving the use of the test gases, a gas metering system was provided as is illustrated in Figure 5.

To provide the large amounts of air necessary to achieve the desired gas dilutions, rotary air pumps of the type used to operate laboratory blast lamps were employed. Each pump provided 1.5 cubic feet of air per minute in continous operation. For this, and all subsequent gas experiments, only glass and Tygon tubing were used to minimize the possibility of odor being imparted to the air or air-gas flow by the lines themselves. All experimental gases were obtained from the Matheson Company, E. Rutherford, N. J., in high pressure cylinders. The following gases were used in 1956: carbon dioxide, commercial grade, 99.5 percent minimum purity, and methane, technical grade, 95.0 percent minimum purity. In 1957, in addition to these two gases hydrogen sulfide, purified grade, 99.5 percent minimum purity, was also used. To obtain the desired concentrations, the specific gas to be used was metered out of the compressed gas cylinders through a "Rotameter" type flowmeter (obtained from the Emil Greiner Co., New York).

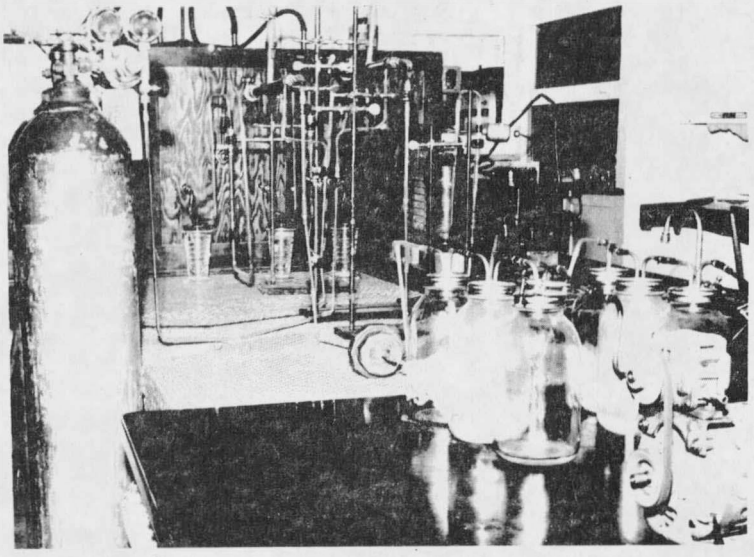


Fig. 5. Overall view of gas metering apparatus, showing rotary air-pump and line filters in the foreground. These filters removed all oil vapor from the pump air flow.

The air flow from the pump was measured through a larger flowmeter. The gas and air were then led into a mixing chamber consisting of a series of washers soldered inside of a length of copper tubing. The Venturi-tube action of these washers upon the air-gas flow created enough turbulence to mix them thoroughly. The excess air-gas mixture not needed for the experiments was removed through a "bleed-off" line in the mixing chamber. Fig. 6 shows the gas metering and mixing chamber equipment. The air-gas mixture flow to be used was then regulated through a needle valve, then led through another flowmeter to measure the amount passing through the chamber line leading to the olfactometer chamber. After passing through a Fisher-Milligan type gas washing bottle to humidify the flow of air-gas mixture (or air as the case might be) it was introduced into the olfactometer

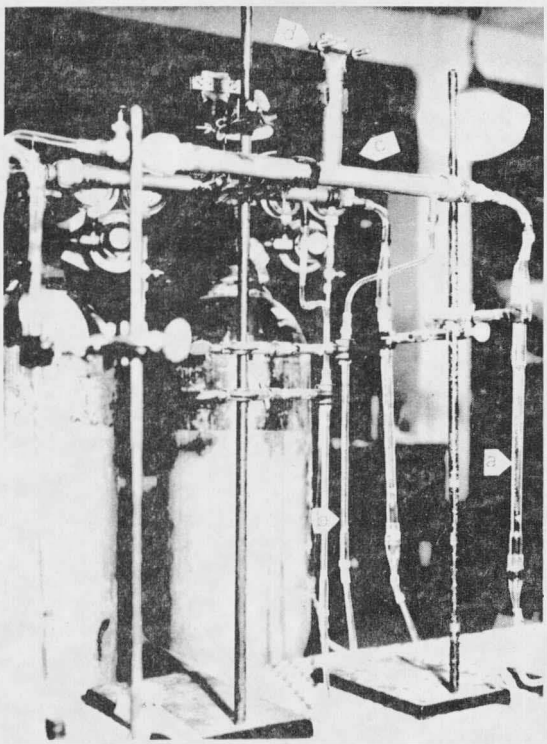


Fig. 6. Details of flowmeter and mixing chamber apparatus
a. Airline flowmeter
b. Gas line flowmeter
c. Mixing Chamber
d. "Bleed-off" line

chamber through a Buchner funnel as described previously. To prevent a buildup of gases within the chamber, an exhaust funnel was located over each Buchner funnel. In use, these exhaust funnels were connected to a pump, and were regulated by use of another flowmeter to exhaust air from the chamber at a slightly faster rate than the air-gas mixtures were introduced. For the one experiment in 1956 in which gases were used, two funnels of the four were provided with air-gas mixtures at a rate of 100 liters per hour per funnel, one was a control with pure air at the 100

liters per hour rate, and the fourth was also a control with no air passing through it. The exhaust rate was 150 liters per hour per exhaust funnel.

For each 1956 experiment, mosquitoes were captured with an aspirator after they had engorged from a horse. These mosquitoes were then transferred to a conditioning cage measuring 18 X 18 X 18 inches constructed from plywood. A "false floor" separated the cage proper from an 18 X 18 X 4 inch chamber where water evaporation trays were placed to keep the relative humidity in the cage at about 75 to 80 percent. This cage was provided with a sleeve opening for manipulating the mosquitoes, and glass observation windows front and back. For nutrients, the mosquitoes had access at all times to Petri dishes containing cellucotton pads saturated with 10 percent sucrose solution. The mosquitoes were conditioned an average of three days (range two to six days).

The mosquitoes were introduced into the olfactometer and were left in the olfactometer for varying lengths of time (depending on the individual tests), usually until all had died. The toweling was then removed from the funnels and the eggs were counted under 13X magnification with a binocular microscope.

Due to low egg numbers produced in this preliminary design chamber, it was decided to modify it for the 1957 work, with the idea in mind of increasing the percentage of mosquitoes that would oviposit in the test chamber.

For the 1957 work, the outside chamber was modified to gain better humidity and temperature control. For a humidifying system a Minneapolis-

Honeywell "snap-action" type human hair humidity control with a 2 percent relative humidity differential was connected through a relay to control a steam vaporizer unit. This unit discharged its vapor into the air circulation system of the constant temperature chamber. For more precise control of temperature, the pressure control unit formerly used on the refrigerator unit was replaced with a special Minneapolis-Honeywell mercury switch control, having a 1/2° F. differential. This was connected through a relay and motor starting unit to the refrigerator unit, and it was found that by connecting the air circulation fan to run only when the refrigerator unit was running the temperature variation could be held to a 1° or 1 1/2° F. range. By removing the cover of the humidity control for better air stream contact it was possible to control the relative humidity variation to not more than 5 percent.

Another change in procedure for the 1957 work was replacement of the large conditioning cage used for the 1956 work with small screen top cages 2 X 2 X 10 inches in size. The mosquitoes were transferred from the aspirator to these cages as they were caught, and then the cages were placed in covered refrigerator crisper trays for the conditioning period. A piece of water saturated cellucotton in each tray sufficed to keep relative humidity between 60 and 70 percent, and a small piece of cellulose sponge saturated with 10 percent sucrose solution was laid on the screen top of each cage to provide food and moisture for the mosquitoes. This method of conditioning the mosquitoes was found to be superior to that used for the 1956 olfactometer tests because the mosquitoes had to be handled only two

instead of three times as in the 1956 method. This led to a corresponding drop in mortality over the 1956 work, but still a fairly large proportion of the females captured died during the conditioning period, presumably from some slight injury from handling when the abdomen was distended with blood.

Since many of the mosquitoes in the large chamber had been observed to sit quietly on or near the floor level of the chamber, or at most make only short walks or flights, it appeared that many of them were not contacting the elevated funnels. Accordingly, because of this apparent lack of orientation to the wet cloths (except by accidental contact), a smaller chamber measuring 40 X 11 1/2 X 6 inches was constructed in such a manner that the cloths over the funnels were flush with the floor of the chamber. This was accomplished by cutting holes just slightly smaller than the diameter of the funnels in the sheet metal floor of the chamber, so that the chamber "rested" on the funnels. Fig. 7. illustrates this chamber. The gas metering and exhaust systems were essentially the same as were described for the chamber used in 1956.

The modified chamber was found to be much more effective than the preliminary chamber in getting increased oviposition. However, since each experiment required several days and only one constant temperature chamber was available, the design of the olfactometer chamber was modified again so that more replicates could be accomplished in a given time. This was done by building four small "replicate" olfactometer chambers 5 1/2 X 5 1/2 X 1 1/2 inches in size, each of which occupied a location in the

outer chamber formerly occupied by a Buchner funnel. Each of these four "replicate" chambers was in turn provided with four small glass funnels 2 inches in diameter. Fig. 8. illustrates these chambers. Since these funnels were too small to allow placing water reservoirs under the funnel cloths to keep the cloths moist, it was found necessary to supply water to the cloths by means of cellulose sponge wicks from pint jars outside of the chambers. The gas metering apparatus was modified for these "replicate" chambers by dividing the control air line and each air-gas chamber line into four smaller lines, one for each chamber. The gas washing bottles were replaced with capillary inlet test tubes, similar to those used by Wallis and Wilde (1957) in metering soil carbon dioxide extractions. Each of the four olfactometer chambers was provided with four funnels (three with air or air-gas mixtures passing through them and one control with no movement of air). One metering test tube was necessary for each line carrying air or air-gas mixture. By means of a tubing clamp for adjustment of flow, the metering test tube on each line was made to perform as a flowmeter. This was done by adjusting the tubing clamps on each metering test tube so that a like number of bubbles per unit of time issued from each tube, thus the flow from each air-gas chamber line could be divided into four equal parts and introduced into their respective funnels in the four olfactometer chambers. Fig. 9 illustrates these metering tubes in use.

New square exhaust funnels were made to fit the top of each "replicate" olfactometer chamber, otherwise the exhaust system was the same as before.

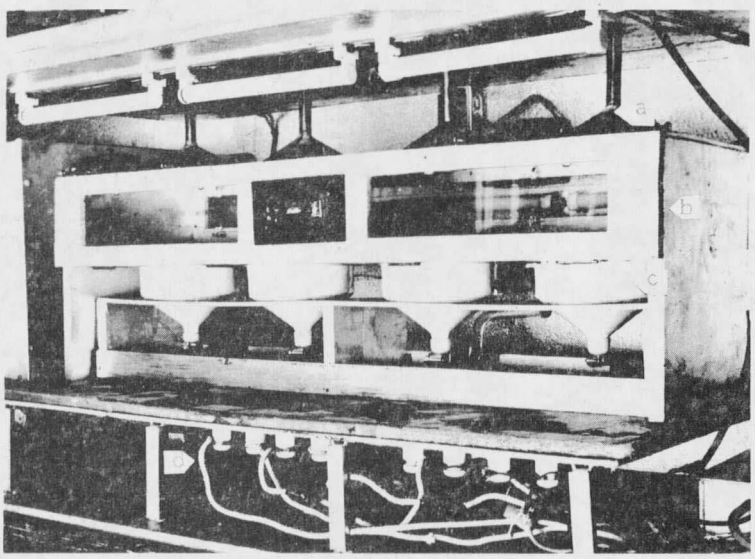



Fig. 7. Modified olfactometer chamber used in 1957 tests.
a. Exhaust funnel
b. Olfactometer chamber
c. Buchner funnel
d. Constant temperature chamber heating apparatus

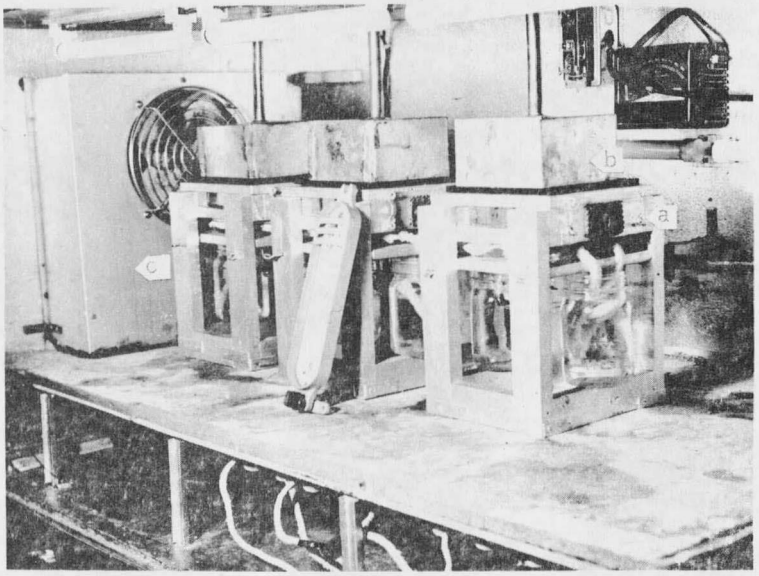



Fig. 8. "Replicate" olfactometer chambers used in 1957 tests.
a. Olfactometer chamber
b. Exhaust funnel
c. Constant temperature refrigerator coils and fan

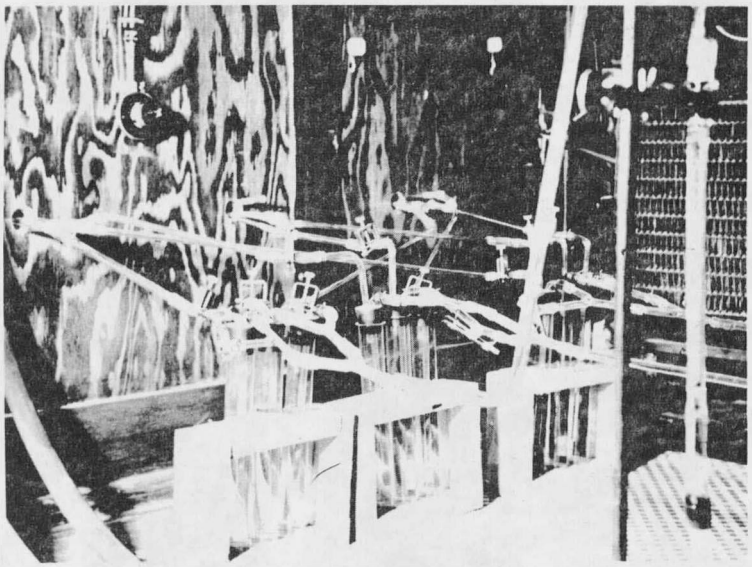


Fig. 9. Details of metering test tubes.

Since Beckel (1955) and others have demonstrated preference for dark rough surfaces for oviposition in certain other mosquito species, several types of cloth were used for the oviposition sites in the 1957 olfactometer tests. These were as follows: brown toweling (rough, course-weave muslin) experiment 1, gray toweling (same type of material as brown toweling) experiment 2, brown corduroy (16 ribs per inch) experiment 5, brown "knob" linen, experiment 6; white cotton plissé, experiments 3, 4, 7, 8, and 9; and white "waffle-weave" pique, experiments 10 and 11.

In 1957, it was decided to experiment with hydrogen sulfide as another possible oviposition stimulus. In the course of experimenting with hydrogen sulfide in the olfactometer chambers, it was discovered that over a period of time small line leaks caused a buildup of this gas in the outer (contant temperature) chamber to the point where it was thought it might

be influencing the mosquitoes. Also leaks from the pressure regulator on the compressed hydrogen sulfide cylinder made working conditions in the laboratory hazardous, since this is a very toxic gas. Consequently a special hydrogen sulfide exhaust chamber, measuring 36 X 36 X 36 inches, was made to enclose the cylinder and all metering lines as well as one olfactometer chamber. This special exhaust chamber was equipped with a double centrifugal ("sirocco") fan exhaust blower, that removed the contaminated air to the exterior of the laboratory. This blower, in connection with stepping up the chamber exhaust funnel suction, was found to keep the hydrogen sulfide level down to a workable level inside the exhaust chamber, although the odor of the gas was still discernable when the door to the exhaust chamber was opened. Due to time and equipment limitations it was not possible to install humidity and temperature controls in this exhaust chamber. One of the four small "replicate" olfactometer chambers built for the constant temperature chamber was used for the hydrogen sulfide experiments in this exhaust chamber. Therefore, just three olfactometer chambers were used for the last two carbon dioxide and methane experiments in the constant temperature chamber. For these, all lines were divided into three parts instead of four as previously described.

For all experiments carried out in 1957 in the constant temperature chamber (experiments 1-9) the temperature range was 75° F. (plus or minus 1/2°) and the relative humidity range was 60 to 65 percent. In the case of the two experiments carried out in the hydrogen sulfide exhaust chamber (experiments 10 and 11) it was not possible to control the temperature and

relative humidity. Therefore for experiment 10 the temperature range was 78° to 88° F. with the relative humidity range being from 30 to 60 percent. For experiment 11, the temperature ranged from 70° to 80° F. and the relative humidity varied from 30 to 50 percent. All temperatures were recorded by Taylor maximum-minimum thermometers, and relative humidities were checked with a Fisher Laboratory Standard hygrometer.

The average number of eggs laid per female in the olfactometer tests was relatively low, when compared with the number a female is capable of laying. Therefore, it was decided to give those mosquitoes still in "healthy" condition at the completion of each experiment a chance to oviposit in a pint mason jar as described for the 55° F. test conducted in 1956. One of these "jar" tests was done for each experiment for experiments 2 through 11.

It was not possible to use the same number of mosquitoes for each experiment, since the availability of specimens was affected by the influence of weather conditions on collecting. Several consecutive days of cool, cloudy, and windy weather (a situation which was all too common) made collection of sufficient individuals for an experiment difficult. Also, mortality varied between different "batches" of mosquitoes during the conditioning period. This made it impossible to predict how many of a group of mosquitoes collected several days before would still be alive and in suitable condition for an experiment.

## Results

Table IV shows the results of the 1956 temperature, humidity and olfactometer experiments. As far as could be determined by observation, little difference in the behavior of the mosquitoes could be noted in varying the relative humidities between 60 and 90 percent. It is realized that no gradient was available for preference in any one experiment, but still no recognizable behavior differences were noted that could be attributed to difference in relative humidity. One difficulty was encountered, however, in working with relative humidities of from 80 to 92 percent. With the increased amount of water vapor present in the air in this range, condensation occurred within the olfactometer chamber, particularly on the observation window. Some mosquitoes that flew against the window were trapped in the condensation and some laid eggs during their struggles. Temperature variation did cause an effect on the oviposition behavior of the mosquitoes. The upper temperature limit at which oviposition will occur was not determined, since there were eggs laid at 95° F., the highest temperature tested. However, it was noted that mortality occurred quickly at this temperature, and only a few of the mosquitoes in the test survived for more than 36 hours. The data in experiment 9, Table IV, indicate that A. nigromaculis females held at a temperature of 55° F. will not oviposit, and that this is, or is near, the lower limit of temperature for oviposition.

At the termination of the 55° F. experiment the 45 surviving females placed in the pint jar at room temperature began to show typical oviposition

Table IV. Results of 1956 experimental chamber tests.

| Exp. No. | Duration in Hours | No. Females Used | Temperature Range | Relative Humidity Range | Egg Distribution | | | | | Av. No. Eggs per Female |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Funnels[1] | | | | Other[2] | |
| | | | | | A | B | C | D | | |
| 1 | 86 | 105 | 76°-77° F. | 63-66% | - | - | - | - | - | - |
| 2 | 84 | 55 | 74°-79° F. | 66-73% | 135 | 32 | 108 | 25 | 54 | 6.4 |
| 3 | 94 | 50 | 79°-85° F. | 60-64% | 21 | 109 | 72 | 196 | - | 8.0 |
| 4 | 74.5 | 50 | 76°-79° F. | 76-82% | 6 | 1 | 81 | 20 | 39 | 2.9 |
| 5 | 53 | 100 | 87°-90° F. | 70-92% | - | 7 | - | 39 | - | 0.5 |
| 6 | 96 | 125 | 74°-78° F. | 72-84% | - | 13 | 52 | 123 | 52 | 1.9 |
| 7 | 96 | 50 | 78°-81° F. | 80-86% | 54 | 166 | 153 | 31 | 92 | 8.8 |
| 8 | 157 | 150 | 60° (±2° F.) | 72-80% | - | 4 | 2 | - | 25 | 0.2 |
| 9 | 114 | 150 | 55° (±2° F.) | 72-77% | - | - | - | - | - | - |
| 10 | 148 | 150 | 72°-78° F. | 62-74% | 198 | 90 | 8 | - | 135 | 2.8 |
| 11 | 96 | 150 | 95° (±1.5° F.) | 79-87% | 27 | 326 | 125 | 105 | 36 | 4.1 |
| 12[3] | 60 | 75 | 85° (±1.5° F.) | 82-85% | 37 | 115 | 105 | 48 | - | 4.1 |

[1]Funnels were identified (A, B, C, D) left to right in the chamber.

[2]"Other" indicates eggs laid within the chamber at other locations than the funnel cloths; i.e., on the sugar solution pads or moist areas resulting from condensation of atmospheric moisture, etc.

[3]Experiment 12 was an olfactometer test utilizing the following test substances:
Funnel A - 2:1000 methane-air mix at 100 liters/hr.
Funnel B - 2:1000 carbon dioxide-air mix at 100 liters/hr.
Funnel C - control-air flow at 100 liters/hr.
Funnel D - control-no air flow
Exhaust rate 150 liters/hr./funnel

behavior almost immediately, and within thirty minutes after being placed in the jar several of the mosquitoes were seen in the act of ovipositing. Within two days a total of 3,441 eggs were laid in the jar or an average of 76.4 eggs per female. This indicates that the mosquitoes held at 55° F. were either incapable of, or refused to initiate, the act of oviposition, even though they contained well matured eggs ready to deposit. The 1956 gas experiment data is discussed with the 1957 data.

The results of the 1957 olfactometer tests are shown in Table V. As can be seen from the column "Av. No. eggs per female in test," there were considerable increases in egg numbers laid in the 1957 olfactometer chambers, when compared to the 1956 experimental chamber results shown in Table IV. However, it is apparent from these data that something was still lacking to bring about complete oviposition since large numbers of eggs were laid in the "jar" tests conducted with the survivors of the experiments. Observation of the mosquitoes' behavior when in these jars indicated that the almost immediate oviposition activity noted may be the result of the close association of individuals in the jar. The jostling that resulted when the mosquitoes collided with each other in the small confines of the jar seemed to prevent them from lapsing into a quiescent state. (This state was often noted in the olfactometer, in which an individual would just perch on the side of the chamber for hours at a time without moving.) The stimulus for complete oviposition was apparently still lacking though, because many of the mosquitoes that died in the jars still contained eggs.

Table V. 1957 Olfactometer Tests

| Exp. No. | Duration in Hours | No. of Females Used | Concentrations and Flow Rate of Test Substances in Liters per Hour | Flow Rate of Exhaust in Liters per Hour | Egg Count - By Choice Offered | | | | Av. No. Eggs per Female In Test | Experiment "Jar" Tests | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Methane | Carbon Dioxide | Control-Air Flow | Control-No Air Flow | | No. of Females | No. of Eggs Laid | Av. No. Eggs per Female |
| 1 | 107½ | 150 | 1 percent at 35.4 l. | 100 l. | 2459 | 1644 | 2744 | 1072 | 52.8 | - | - | - |
| 2 | 136 | 94 | 5 percent at 100 l. | 200 l. | 637 | 818 | 825 | 787 | 32.4 | 31 | 1,827 | 52.0 |
| 3 | 96 | 100 | 10 percent at 50 l. | 100 l. | 576 | 1312 | 620 | 535 | 30.4 | 43 | 2,939 | 68.3 |
| 4 | 96 | 200 | 10 percent at 50 l. | 100 l. | 574 | 492 | 729 | 254 | 10.2 | 189 | 5,868 | 31.0 |
| 5 | 72 | 250 | 20 percent at 25 l. | 50 l. | 1328 | 2075 | 2639 | 976 | 28.1 | 218 | 12,121 | 56.6 |
| 6 | 96 | 100 | 1 percent at 25 l. | 50 l. | 554 | 919 | 284 | 619 | 23.8 | 82 | 7,768 | 94.7 |
| 7* | 72 | 50** | 1 percent at 6.2 l. | 25 l. | 389 274 259 205 | 234 398 214 219 | 270 214 210 320 | 323 398 238 223 | 21.3 | 136 | 7,102 | 52.2 |
| 8 | 96 | 30 | 50 percent at 8.4 l. | 20 l. | 329 272 79 - | 261 351 193 - | 380 201 238 - | 329 234 144 - | 33.5 | 90 | 2,701 | 30.0 |
| 9 | 96 | 50 | 100 percent at 4.2 l. | 20 l. | 451 288 213 - | 312 245 136 - | 425 440 460 - | 260 209 402 - | 25.6 | 98 | 2,958 | 30.2 |
| | | | | | Hydrogen Sulfide | Hydrogen Sulfide | Control-Air Flow | Control-No Air Flow | | | | |
| 10*** | 96 | 50 | 1:4,000 at 18 l. | 180 l. | 7 | 43 | 466 | 253 | 15.3 | 19 | 1,210 | 63.6 |
| 11 | 120 | 50 | 1:20,000 at 18 l. | 180 l. | 222 | 60 | 49 | 76 | 8.1 | 27 | 1,347 | 49.9 |

* Experiments 7-9 were conducted in the small "replicate" olfactometer chambers.

** In the "replicate" olfactometer and hydrogen sulfide tests, "No. of Females Used" refers to the number in each of the "replicate" chambers.

*** Experiments 10 and 11 were conducted in the hydrogen sulfide exhaust chamber.

Examination of the data obtained from the egg counts, show no discernible pattern of either oviposition preference or avoidance of the funnels having methane or carbon dioxide flow passing through them. This was true even for experiment 9, Table V, in which these gases were used undiluted.

Hydrogen sulfide, on the other hand, produced pronounced behavior reactions. The first time the use of hydrogen sulfide was attempted in an olfactometer experiment was at a concentration of 1/2 percent. (This experiment is not shown in Table V. due to the fact that it was never completed.) This concentration was far too great and was so toxic that all the mosquitoes in the olfactometer chambers were incapacitated within five minutes, and approximately half of them were killed. One interesting thing about this incident was that many of the mosquitoes ejected masses of eggs from their ovipositors just prior to cessation of struggling. Even some of those overcome by the gas while on the metal surface of the cage floors exhibited this behavior, which was apparently merely a death stress reaction, as described by De Coursey and Webster (1952), and was not connected with a usual oviposition stimulus response. A concentration of one part hydrogen sulfide to 4,000 parts of air (experiment 10, Table V), while not lethal to the mosquitoes, did produce an avoidance reaction which was observed on several occasions. When a mosquito would come into contact with the wet cloth covering one of the hydrogen sulfide test funnels, she would immediately show sign of discomfort and would quickly move off of the cloth. The egg counts show a total of only 50 eggs deposited

on the two hydrogen sulfide test funnels as opposed to 719 deposited on the two control funnels. Furthermore most of the 50 that were deposited on the hydrogen sulfide funnels were in a "clutch" ejected from a female that died on the cloth of one of the funnels. Apparently this mass expulsion of her batch of eggs was a death stress reaction similar to that discussed previously.

At a concentration of one part hydrogen sulfide to 20,000 parts of air (experiment 11, Table V) this avoidance reaction was not noted, and as a matter of fact the largest number of eggs deposited on a test funnel happened to be on a hydrogen sulfide funnel. Apparently the hydrogen sulfide does not repel the mosquitoes at this low a concentration, nor do the data indicate any attraction either. It is true that 222 eggs (the most laid on any funnel in the test) were laid on one of the hydrogen sulfide funnels but only 60 were laid on the replicate hydrogen sulfide funnel. This indicates the apparent random deposition. Apparently a single female laid a large clutch which accounted for most of the 222 eggs on the one hydrogen sulfide funnel.

The use of different cloth types and colors for oviposition sites apparently made little difference to these mosquitoes as far as affecting oviposition goes. Further tests with these differences in color and texture of substrate, which are reported in the section dealing with individual isolations in comparison vials, also indicated that these factors are not of importance in choice of an oviposition site in this mosquito species.

## INDIVIDUAL MOSQUITO ISOLATIONS

### Methods:

This work was begun in the summer of 1956 to obtain information on the time necessary for A. nigromaculis females to mature eggs after a blood meal, and to find the approximate range in numbers of eggs produced by each female. Individual females were isolated in vials as described by Mail (1934).

These vials were 1 1/2 inches in diameter and 3 1/2 inches high. Each was provided with a layer of cellucotton in the bottom which was kept saturated with distilled water. A piece of white filter paper was placed over this to provide an oviposition surface. For food each vial was provided with a raisin suspended on an insect pin to prevent juice from contaminating the filter paper, and a twig to provide a "roost" for the mosquito. The raisins were changed every other day to prevent mold formation. The vials were stoppered with cotton to allow some evaporation and to prevent condensation of water vapor on the walls of the vials. Periodically the filter papers were removed and any eggs present were counted with a binocular microscope. In all cases, following the death of each mosquito, the abdomen was dissected under 13X magnification and all of the unlaid eggs counted.

During the summer of 1957, this isolation procedure was modified to gain information about the ability of a female A. nigromaculis to choose between solutions of different pH, and also to choice between types of substrata. Straight-sided plastic vials 1 1/4 inches in diameter and 3

inches deep were used. To keep the substances separated, two water-tight cups were made by forming heavy aluminum foil around a wooden form. Fig. 10. shows these vials as they were used. The use of a raisin in each vial was discontinued, because even with an alcohol dip treatment, fungus growth from spores on the raisin occurred quickly. As a food source 10 percent sucrose solution was offered on a cotton wad wrapped on a tooth pick and suspended by a thread. This prevented sugar solution contamination of the test substances, and was found to be a satisfactory method of feeding.

The mosquitoes for these tests were blood-engorged females captured from a horse, and conditioned for an average of 5.2 days (range 1-11 days) prior to introduction into the vials. Conditioning was done in small screen top cages measuring 2 X 2 X 10 inches in the manner described in the olfactometer tests methods section.

Alkalinity tests were made of river water, irrigation water diverted from the river, water that had been on a field for several days, and wet soil from several locations in a field. These tests were made with a cresol red - phenol red indicator kit of a type used for testing city water supplies. The alkalinity range was found to be from pH 8.3 for some soil surface samples to about pH 7.0 for some stagnant field water samples. Records obtained from the Chinook, Montana city water engineer showed that the river water pH varied from 7.4 to 8.4, depending on how much irrigation return, rich in dissolved soil salts, was being discharged into the river upstream.

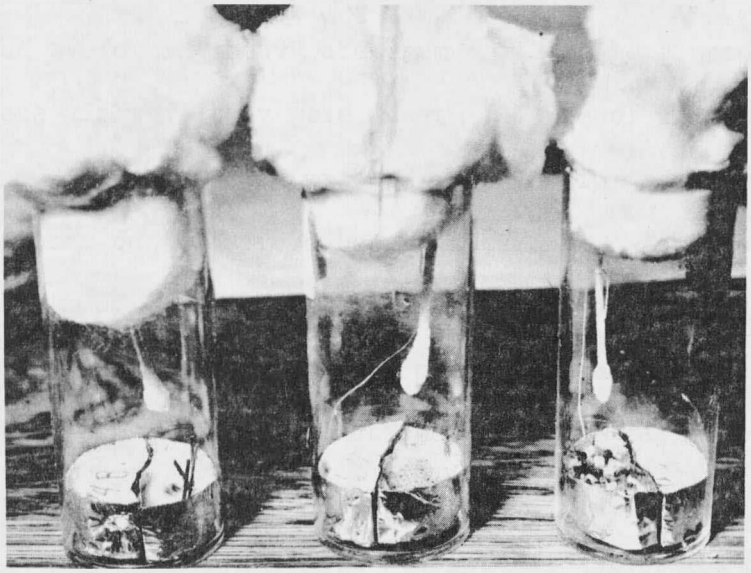


Fig. 10. Substance comparison vials used in 1957.

Accordingly, it was decided to test the ability of the ovipositing females to distinguish between differences in alkalinity in a range of pH 6.6 to 8.3, and to also see if there was some substance present in the field soil, or irrigation water which was possibly influencing the choice of oviposition sites. Table VI. shows the various test substances used, as well as the "texture" test materials used to test if mechanical variations in the surface of the substrate influenced oviposition.

Unless otherwise specified, the test solutions were introduced from a pipette into a pad of cellucotton cut to fit the shape of the test cup and extending to within about 1/8 inch below the edge of the cup. A separate pipette was used for each solution to avoid contamination. A new cellucotton pad and new solution was used for each mosquito. With the

Table VI. Substances used in comparison vials - 1957.

| Substance | Code Symbol | Remarks |
|---|---|---|
| pH 6.6 Solution | 6.6 | A buffered sodium phosphate - citric acid solution. |
| pH 7.0 Solution | 7.0 | A buffered sodium phosphate - citric acid solution. |
| pH 7.6 Solution | 7.6 | A buffered sodium phosphate - citric acid solution. |
| Distilled Water | Dis. | pH 7.0(+). Used in all cups unless indicated otherwise. |
| Tap Water | Tap | From Chinook water system - pH 7.2 |
| pH 6.6a Solution | 6.6a | An unbuffered solution prepared from distilled water and sufficient 2N $H_2SO_4$ solution to achieve the desired pH. |
| pH 7.0a Solution | 7.0a | An unbuffered solution prepared from distilled water and sufficient 2N $H_2SO_4$ solution (only about 2 drops) to achieve the desired pH. |
| pH 7.6a Solution | 7.6a | An unbuffered solution prepared from distilled water and sufficient 2N NaOH solution to achieve the desired pH. |
| Field Water | Fld. | From a field 6 miles N.W. of Chinook. Had been standing several days - pH 7.8 |
| Ditch Water | Dit. | From irrigation canal 6 miles N.W. of Chinook (taken Aug. 15, 1957) - pH 7.8 |
| Sterilized Water | S.W. | Above ditch water but boiled for 5 minutes. |
| pH 8.3 Solution | 8.3 | Ditch water (taken Sept. 9, 1957) |
| pH 6.6b Solution | 6.6b | 8.3 Ditch water and sufficient 2N $H_2SO_4$ to achieve the desired pH. |
| Tooth Picks | T.P. | Four short lengths of tooth picks placed upright in the cellucotton of one cup to simulate grass stems as perches. |
| Filter Paper | F.P. | Filter paper placed over the cellucotton in a cup. |
| Cellucotton | C.C. | Cellucotton in a cup - no filter paper on it. |
| Brown Toweling | B.T. | Rough, coarse, unbleached muslin cloth. |
| White Plisse' | W.P. | Crinkled cotton crepe cloth. |
| Brown Corduroy | B.C. | Dark brown corduroy cloth - 16 ribs per inch. |
| Soil | Sl. | Wet soil from surface of field 6 miles N.W. of Chinook - pH 8.0. Covered with filter paper. |
| Sterilized Soil | S.S. | Same soil but heated 30 minutes in 500° F. oven. Covered with filter paper. |
| Sand | Sd. | "B.B."-sized washed quartz particles spread on filter paper to provide uneven surface. |

exception of the "texture" tests the cellucotton was covered with filter paper. The soil and the field and ditch water tests were undertaken to see if there was any substance, in solution or evolved as a gas, that was possibly bacterially produced, and which might be affecting oviposition choice. Experiments 37 and 38 were designed to test whether or not the filter paper itself was influencing oviposition, and also to determine whether or not an upright "perch" (as would be provided in nature by grass stems) was a stimulus to oviposition. To simulate the grass stems, four short lengths of toothpick were inserted into the cellucotton of one cup in each vial (see left vial in Fig. 10). In experiment 38 all cups were covered with filter paper, while in experiment 37 the cellucotton was left uncovered. The different types of cloth were used to determine if different colors or textures of surface affected oviposition.

All mosquito transfers were accomplished by the use of a glass aspirator, and care was taken to avoid use of any mosquitoes that appeared to be injured in any way. All conditioning and all tests were done in a laboratory where the temperature range was from 65° F. to 85° F. No special attempt was made to regulate lighting which was artificial light supplemented by some natural light from windows during the day, and darkness at night.

Each vial was observed three times a day, and each individual test was concluded only with the death of the mosquito. The cups were then removed, the eggs counted, and the mosquito was dissected to determine the number of eggs remaining in the abdomen at time of death. Due to time limitations it

was impossible to count the individual eggs in each post mortem, so a visual estimation of the number of eggs was made, and recorded as "none", "few", "some", or "many".

Several individuals from each estimation class were set aside for accurate counting, and it was found that in these the egg range in the "few" class was from 1 to 17 eggs, in the "some" class 19 to 61 eggs and in the "many" class 72 to 181 eggs.

## Results

Table VII. shows the results of the 45 individual isolations made in 1956. Only four (or 8.9 percent) of the 45 showed no egg development at all. Ten (or 22.2 percent) of the remaining 41 developed eggs, but did not lay any, retaining their clutches in their abdomen until death. Thirty-one (or 68.8 percent) of the 45 deposited eggs. Of these 15 (or 33.3 percent) laid their entire clutches and 16 (or 35.6 percent) retained some (range 5-190) eggs in their abdomens. Of the 41 that either laid eggs or had eggs in their abdomen at death, or both, the average number of eggs found was 127.6 (range 2-225), but counting only the eggs actually laid the average was 94.5 (range 1-225) for the 31 females participating. The shortest time after the blood meal that passed prior to oviposition was 36 hours and the longest was 384 hours (16 days). The average length of life after capture was 13.6 (range 3 3/4 to 28) days.

Since these mosquitoes were captured wild, there was no assurance that all would be capable of oviposition because some may have oviposited before capture or may not have mated. However, the above figures show that about

Table VII. Results of 1956 individual female isolations.

| No. Days Lived After Capture | Hours After Blood Meal to Oviposition | No. Eggs Laid | Eggs Retained at Death | Total Eggs per Female |
|---|---|---|---|---|
| 5 | - | - | - | - |
| 8 | - | - | - | - |
| 8 | - | - | - | - |
| 28 | - | - | - | - |
| 5 1/2 | - | - | 13 | 13 |
| 16 | - | - | 92 | 92 |
| 18 | - | - | 126 | 126 |
| 8 1/2 | - | - | 56 | 56 |
| 4 1/2 | - | - | 101 | 101 |
| 7 | - | - | 175 | 175 |
| 7 | - | - | 26 | 26 |
| 18 | - | - | 138 | 138 |
| 27 | - | - | 175 | 175 |
| 21 | - | - | 181 | 181 |
| 6 | 36 | 97 | 5 | 102 |
| 5 1/2 | 72 | 27 | 37 | 64 |
| 15 1/2 | 77 | 187 | - | 187 |
| 3 3/4 | 96 | 31 | 51 | 82 |
| 8 | 96 | 112 | - | 112 |
| 5 1/2 | 100 | 3 | 40 | 43 |
| 27 | 120 | 155 | - | 175 |
| 5 | 120 | 7 | 150 | 157 |
| 11 | 120 | 103 | - | 103 |
| 5 | 120 | 38 | 12 | 50 |
| 7 | 150 | 100 | - | 100 |
| 21 | 150 | 86 | 41 | 127 |
| 18 | 168 | 144 | 35 | 179 |
| 10 | 180 | 136 | - | 136 |
| 9 1/2 | 185 | 129 | 30 | 159 |
| 8 | 187 | 114 | - | 114 |
| 28 | 192 | 195 | - | 195 |
| 9 1/2 | 210 | 93 | 64 | 157 |
| 15 1/2 | 240 | 8 | 161 | 169 |
| 15 | 280 | 1 | 190 | 191 |
| 13 | 288 | 80 | 45 | 125 |
| 21 | 290 | 59 | - | 59 |
| 21 | 300 | 167 | - | 167 |
| 15 | 312 | 49 | 175 | 224 |
| 15 | 336 | 169 | - | 169 |
| 15 | 355 | 158 | - | 158 |
| 16 1/2 | 360 | 99 | 111 | 210 |
| 20 | 360 | 2 | - | 2 |
| 20 | 360 | 225 | - | 225 |
| 25 | 360 | 71 | - | 71 |
| 17 | 384 | 84 | 51 | 135 |

90 percent of the mosquitoes obtained in this way can be expected to produce at least some eggs. Approximately one-fourth of the mosquitoes refused to oviposit even though their eggs were well developed at the time of their death. This suggests the possibility that some stimulus other than the presence of water may play a part in inducing oviposition.

Table VIII. shows the results obtained from the 276 females isolated in the 1957 comparison vial experiments, arranged by the 44 choices offered. The number of females offered each choice in individual vials varied from 1 to 17. It is realized that too few tests of certain choices were included to be of comparison value. These results were included because these data form a part of the totals obtained from the entire series. The terms "viable" and "inviable" were used to designate the conditions of the eggs upon oviposition. The inviable eggs were those which failed to attain normal pigmentation. Quite often these eggs were not turgid and some were deformed and smaller than the normally pigmented "viable" egg. Apparently all non-pigmented eggs were "inviable", since representative samples set aside for hatching trials showed no hatch, while the normally pigmented eggs showed a high percentage of hatch. Woke (1955) noted similar defective eggs in A. aegypti.

The number of eggs laid in these tests varied from 1 to 228. For purposes of comparison it was arbitrarily decided to group those mosquitoes laying 1-10 eggs, 11-25 eggs, etc., as designated in Table VIII. The "visual estimation class" columns refer to the post mortem examinations which are explained in the methods section. The last column in Table VIII

Table VIII. Oviposition results from 276 females isolated in comparison vials - 1957

| Exp. No. | Choice Offered | No. In Test | Percent Laying No Eggs | Percent of Females Laying: Only Viable Eggs | Only Inviable Eggs | Viable & Inviable Eggs | Percent of Females Laying: 1-10 Eggs | 11-25 Eggs | 26-50 Eggs | 51-75 Eggs | 76-100 Eggs | 101-150 Eggs | 151-288 Eggs | Post Mortem Egg Condition by Percent: None | Few | Some | Many | Percent Showing No Egg Development |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 7.6 -7.6 | 10 | 30.0 | 10.0 | 60.0 | - | 50.0 | - | - | - | - | 20.0 | - | 30.0 | - | 20.0 | 50.0 | - |
| 2 | 7.0 -7.0 | 7 | 28.6 | - | 57.2 | 28.6 | 14.2 | - | - | 42.8 | 14.2 | - | - | - | 14.3 | - | 85.7 | - |
| 3 | 6.6 -6.6 | 5 | 20.0 | - | 40.0 | 40.0 | 20.0 | - | 20.0 | - | 20.0 | 20.0 | - | - | 20.0 | 20.0 | 60.0 | - |
| 4 | 7.6 -7.0 | 10 | 50.0 | 10.0 | 40.0 | - | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | - | - | 10.0 | 10.0 | 20.0 | 60.0 | 10.0 |
| 5 | 6.6 -7.6 | 8 | 25.0 | - | 37.5 | 37.5 | - | 25.0 | 37.5 | 12.5 | - | - | - | 12.5 | - | - | 87.5 | - |
| 6 | 6.6 -7.0 | 8 | 37.5 | - | 50.0 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | - | - | 25.0 | 25.0 | 12.5 | 37.5 | 12.5 |
| 7 | 7.6 -Dis. | 9 | 11.1 | 66.6 | - | 22.2 | 11.1 | - | 33.3 | 22.2 | - | 11.1 | 11.1 | 88.9 | - | - | 11.1 | - |
| 8 | 7.6 -Tap | 3 | 33.3 | 33.3 | - | 33.3 | - | - | - | 33.3 | - | - | 33.3 | 66.6 | - | - | 33.3 | - |
| 9 | 7.0 -Tap | 4 | - | 25.0 | - | 75.0 | 25.0 | 50.0 | - | - | - | 25.0 | - | 25.0 | - | - | 75.0 | - |
| 10 | 7.0 -Dis. | 2 | 50.0 | - | - | 50.0 | - | - | - | - | 50.0 | - | - | 50.0 | - | - | 50.0 | - |
| 11 | 6.6 -Dis. | 6 | 33.3 | 16.6 | - | 50.0 | - | 16.6 | - | 33.3 | - | 16.6 | - | 50.0 | - | - | 50.0 | - |
| 12 | 6.6 -Tap | 5 | 40.0 | 20.0 | 20.0 | 20.0 | 20.0 | - | 20.0 | - | 20.0 | - | - | - | 20.0 | - | 80.0 | - |
| 13 | 7.6A-7.6A | 8 | 50.0 | 50.0 | - | - | 37.5 | - | - | - | - | 12.5 | - | 25.0 | - | - | 75.0 | 12.5 |
| 14 | 7.6A-7.0A | 6 | 66.6 | 16.6 | - | 16.6 | - | - | - | - | - | 16.6 | 16.6 | 33.3 | - | 16.6 | 50.0 | - |
| 15 | 6.6A-6.6A | 4 | 25.0 | 50.0 | - | 25.0 | 25.0 | - | - | - | - | 50.0 | - | 75.0 | - | - | 25.0 | 25.0 |
| 16 | 7.0A-7.0A | 2 | 50.0 | 50.0 | - | - | - | - | - | - | - | 50.0 | - | 50.0 | - | - | 50.0 | - |
| 17 | 6.6A-Tap | 2 | 50.0 | 50.0 | - | - | - | - | - | - | - | - | 50.0 | 50.0 | - | - | 50.0 | - |
| 18 | 6.6A-Dis. | 1 | - | 100.0 | - | - | - | - | - | - | 100.0 | - | - | - | - | - | 100.0 | - |
| 19 | 7.0A-Tap | 3 | 33.3 | 33.3 | - | 33.3 | 33.3 | - | 33.3 | - | - | - | - | 33.3 | - | - | 66.6 | - |
| 20 | 7.0A-Dis. | 3 | 66.6 | 33.3 | - | - | - | - | 33.3 | - | - | - | - | 66.6 | - | - | 33.3 | 33.3 |
| 21 | 6.6A-7.6A | 4 | 50.0 | 25.0 | - | 25.0 | - | 25.0 | - | - | 25.0 | - | - | - | 50.0 | 25.0 | 25.0 | - |
| 22 | 6.6A-7.0A | 7 | 57.1 | 42.8 | - | - | - | - | 14.3 | - | - | 14.3 | 14.3 | 42.9 | - | - | 57.1 | - |
| 23 | 7.6A-Dis. | 4 | 50.0 | 50.0 | - | - | - | - | - | 25.0 | - | 25.0 | - | 25.0 | - | 25.0 | 50.0 | - |
| 24 | 7.6A-Tap | 5 | - | 80.0 | - | 20.0 | - | - | - | 20.0 | 60.0 | 20.0 | - | 80.0 | - | 20.0 | - | - |
| 25 | S.W.-Fld. | 12 | 25.0 | 50.0 | - | 25.0 | 16.6 | - | 8.3 | - | 16.6 | 25.0 | 8.3 | 33.3 | - | 8.3 | 58.3 | - |
| 26 | Fld.-Dis. | 14 | 28.6 | 42.8 | - | 28.6 | 7.1 | 7.1 | 14.3 | 14.3 | 7.1 | 14.3 | 7.1 | 35.7 | 14.3 | - | 50.0 | - |
| 27 | Dit.-Dis | 17 | 41.2 | 47.1 | - | 11.8 | 5.9 | - | 17.6 | - | - | 29.4 | 5.9 | 58.8 | - | 17.6 | 23.5 | 17.6 |
| 28 | Dit.-Fld. | 14 | 21.4 | 42.6 | - | 35.7 | 7.1 | 7.1 | 14.3 | 7.1 | 7.1 | 21.4 | 14.3 | 57.1 | 21.4 | - | 21.4 | 14.3 |
| 29 | Tap -Tap | 6 | 50.0 | 33.3 | - | 16.6 | - | - | - | - | 16.6 | 16.6 | 16.6 | 66.6 | - | - | 33.3 | 16.6 |
| 30 | Dis.-Dis. | 7 | 28.6 | 42.8 | - | 28.6 | - | - | 14.3 | 14.3 | - | 14.3 | 28.6 | 71.4 | - | - | 28.6 | 14.3 |
| 31 | 6.6B-6.6B | 3 | 66.6 | - | - | 33.3 | - | - | - | 33.3 | - | - | - | 33.3 | 33.3 | - | 33.3 | - |
| 32 | 6.6B-8.3 | 9 | 66.6 | 22.2 | - | 11.1 | 11.1 | - | 11.1 | - | 11.1 | - | - | 55.5 | - | 11.1 | 33.3 | 33.3 |
| 33 | 8.3 -Dis. | 2 | 100.0 | - | - | - | - | - | - | - | - | - | - | 50.0 | - | - | 50.0 | 50.0 |
| 34 | 6.6B-Dis. | 4 | 25.0 | 75.0 | - | - | 50.0 | - | - | 25.0 | - | - | - | 25.0 | - | - | 75.0 | - |
| 35 | 8.3 -8.3 | 1 | 100.0 | - | - | - | - | - | - | - | - | - | - | - | - | - | 100.0 | - |
| 36 | 6.6B-Tap | 1 | 100.0 | - | - | - | - | - | - | - | - | - | - | - | - | - | 100.0 | - |
| 37 | T.P.-C.C. | 5 | 20.0 | 60.0 | - | 20.0 | 20.0 | 40.0 | - | - | 20.0 | - | - | 60.0 | 40.0 | - | - | 20.0 |
| 38 | T.P.-F.P. | 10 | 40.0 | 40.0 | - | 20.0 | - | - | 30.0 | - | 30.0 | - | - | 50.0 | - | - | 50.0 | 10.0 |
| 39 | B.T.-F.P. | 4 | 50.0 | 50.0 | - | - | - | - | - | 25.0 | - | 25.0 | - | 50.0 | - | - | 50.0 | - |
| 40 | W.P.-F.P. | 2 | 50.0 | 50.0 | - | - | - | - | 50.0 | - | - | - | - | - | - | - | 100.0 | - |
| 41 | B.C.-F.P. | 3 | 33.3 | 33.3 | - | 33.3 | - | - | - | 33.3 | 33.3 | - | - | 66.6 | - | - | 33.3 | - |
| 42 | S.S.-Sl. | 15 | 20.0 | 73.3 | - | 6.6 | - | 6.6 | 26.6 | 13.3 | 20.0 | 13.3 | - | 66.6 | - | 6.6 | 26.6 | - |
| 43 | Sl. -Dis. | 14 | 7.1 | 71.4 | - | 21.4 | 21.4 | 7.1 | 7.1 | 21.4 | 14.2 | 14.2 | 7.1 | 64.3 | 7.1 | - | 28.6 | - |
| 44 | Sd. -F.P. | 7 | 28.6 | 57.1 | - | 14.3 | 28.6 | - | - | 28.6 | - | 14.3 | - | 71.4 | - | - | 28.6 | 14.3 |

shows those females that laid no eggs and had no eggs in their abdomen at post mortem.

Of the total of 276 females tested, 95 (or 34.4 percent) laid no eggs. One hundred and six (or 38.4 percent) of the total laid only viable eggs, 23 (or 8.3 percent) laid only inviable eggs, and 52 (or 18.8 percent) laid both viable and inviable eggs. Comparing the number of eggs laid per female, 31 (or 11.2 percent) laid from one to ten eggs, 14 (or 5.1 percent) laid from 11 to 25 eggs, 32 (or 11.6 percent) laid from 26 to 50 eggs, 28 (or 10.1 percent) laid from 51 to 75 eggs, 27 (or 9.8 percent) laid from 76 to 100 eggs, 34 (or 12.7 percent) laid from 101 to 150 eggs, and 14 (or 5.1 percent) laid from 151 to 228 eggs. As can be seen from these figures, there was rather even distribution in the number of eggs laid, making it difficult to arbitrarily arrive at a satisfactory figure which could be called the "average number of eggs laid".

Considering the post mortem ovary conditions, 122 (or 44.2 percent) of the total had no eggs, 17 (or 6.2 percent) had a few eggs, 16 (or 5.8 percent) had some eggs, and 119 (or 43.1 percent) had many eggs in their abdomens. These figures represent the respective visual estimation classes "none", "few", "some", and "many". From these results it would appear that in the majority of cases, once an individual started laying her eggs she tended to continue until her supply was exhausted, in a sort of "all-or-none" action. Nineteen (or 6.9 percent) showed no egg development, having laid no eggs and showing none in post mortem examination. Subtracting these 19 females from the 95 not laying eggs gives a total of 76

(or 27.5 percent) of the mosquitoes which developed eggs but refused to oviposit.

The analysis of all data obtained on the influence of the various pH, soil, water, and "texture" choices revealed no discernible effect on oviposition behavior with the exception of the buffered sodium phosphate-citric acid solutions. Table IX. shows the egg distributions of the individual mosquitoes that laid eggs in these buffered pH solution experiments compared with a test using distilled water. It can be seen that when a female had only the buffered solutions to choose from, she laid very few (if any) viable eggs, and that when she had a choice between a buffered solution and either distilled or tap water, a definite avoidance of the buffered solution was demonstrated. Figure 11. illustrates a typical avoidance of a buffered solution. The two cups marked 9A and 9B were from a vial comparing the unbuffered pH 6.6 (code 6.6a) solution (cup A) with tap water (cup B). As can be seen, the female in this vial laid eggs freely on both cups. The two cups marked 10A and 10B were from a vial comparing the buffered pH 6.6 solution (cup A) with tap water (cup B). It is evident that the female in this vial clearly avoided the buffered pH 6.6 solution when depositing her eggs. This avoidance is especially evident if the viable egg totals of Table IX. are compared. In some cases eggs were laid on the buffered solutions but these eggs were largely inviable. The possibility exists that the substances in the buffered solutions were toxic to the newly deposited eggs, thus causing them to be inviable. However, since some viable eggs were laid on the buffered solutions, it

Table IX. Egg distribution in buffered pH solutions (experiments 1-12) compared with distilled water (experiment 30) as a control.

| Treatment | | Mosq. | No. Eggs Laid | | | |
|---|---|---|---|---|---|---|
| | | | Cup A | | Cup B | |
| Cup A | Cup B | No. | V** | I* | V** | I* |
| 7.6 | 7.6 | 1 | - | - | - | 2 |
| " | " | 2 | - | 67 | - | 46 |
| " | " | 3 | - | 4 | - | - |
| " | " | 4 | 1 | - | - | - |
| " | " | 5 | - | 63 | - | 59 |
| " | " | 6 | - | 7 | - | 4 |
| " | " | 7 | - | 1 | - | 1 |
| | | Total | 1 | 142 | 1 | 112 |
| 7.0 | 7.0 | 1 | - | 46 | 1 | 19 |
| " | " | 2 | - | 13 | - | 2 |
| " | " | 3 | - | 39 | - | 25 |
| " | " | 4 | 5 | 58 | 4 | 26 |
| " | " | 5 | - | 30 | - | 45 |
| | | Total | 5 | 86 | 5 | 117 |
| 6.6 | 6.6 | 1 | - | 4 | - | - |
| " | " | 2 | 2 | 43 | 9 | 30 |
| " | " | 3 | - | 4 | - | 29 |
| " | " | 4 | 9 | 62 | 9 | 32 |
| | | Total | 11 | 113 | 18 | 91 |
| 7.6 | 7.0 | 1 | - | 34 | - | 30 |
| " | " | 2 | - | 48 | - | 31 |
| " | " | 3 | - | 13 | - | 6 |
| " | " | 4 | 6 | - | - | - |
| " | " | 5 | - | 41 | - | 8 |
| | | Total | 6 | 136 | 0 | 75 |
| 6.6 | 7.6 | 1 | - | 17 | - | 1 |
| " | " | 2 | - | 14 | - | 19 |
| " | " | 3 | - | 37 | - | - |
| " | " | 4 | 6 | 34 | 2 | 12 |
| " | " | 5 | 1 | 2 | 15 | 22 |
| " | " | 6 | 5 | 26 | - | 5 |
| | | Total | 12 | 130 | 17 | 59 |
| 6.6 | 7.0 | 1 | - | 14 | - | - |
| " | " | 2 | - | 48 | - | 21 |
| " | " | 3 | - | 19 | - | 9 |
| " | " | 4 | - | 1 | - | - |
| " | " | 5 | 2 | 53 | 2 | 21 |
| | | Total | 2 | 135 | 2 | 51 |

| Treatment | | Mosq. | No. Eggs Laid | | | |
|---|---|---|---|---|---|---|
| | | | Cup A | | Cup B | |
| Cup A | Cup B | No. | V** | I* | V** | I* |
| 6.6 | Dis. | 1 | 14 | 25 | 99 | 2 |
| " | " | 2 | - | 6 | 69 | - |
| " | " | 3 | 4 | - | 9 | - |
| " | " | 4 | 3 | 9 | 63 | - |
| | | Total | 21 | 40 | 240 | 2 |
| 6.6 | Tap | 1 | 1 | 2 | - | - |
| " | " | 2 | - | 6 | 44 | 4 |
| " | " | 3 | - | - | 86 | - |
| | | Total | 0 | 8 | 130 | 4 |
| 7.0 | Dis. | 1 | - | 30 | 52 | 9 |
| | | Total | 0 | 30 | 52 | 9 |
| 7.0 | Tap | 1 | - | - | 12 | - |
| " | " | 2 | - | 13 | 4 | - |
| " | " | 3 | - | - | 1 | 1 |
| " | " | 4 | 3 | 20 | 84 | 2 |
| | | Total | 3 | 33 | 101 | 3 |
| 7.6 | Dis. | 1 | 3 | - | 72 | - |
| " | " | 2 | 1 | 42 | 60 | 17 |
| " | " | 3 | 10 | 21 | 32 | - |
| " | " | 4 | 13 | - | 32 | - |
| " | " | 5 | 5 | - | 178 | - |
| " | " | 6 | 1 | - | - | - |
| " | " | 7 | 1 | - | 29 | - |
| " | " | 8 | - | - | 40 | - |
| | | Total | 34 | 63 | 443 | 17 |
| 7.6 | Tap | 1 | 1 | 70 | 84 | 6 |
| " | " | 2 | 3 | - | 52 | - |
| | | Total | 4 | 70 | 136 | 6 |
| Dis. | Dis. | 1 | 72 | - | 113 | - |
| " | " | 2 | 74 | 2 | 84 | - |
| " | " | 3 | 33 | 1 | 36 | - |
| " | " | 4 | 103 | - | 12 | - |
| " | " | 5 | 12 | - | 19 | - |
| | | Total | 294 | 3 | 264 | 0 |

* I - Inviable
** V - Viable

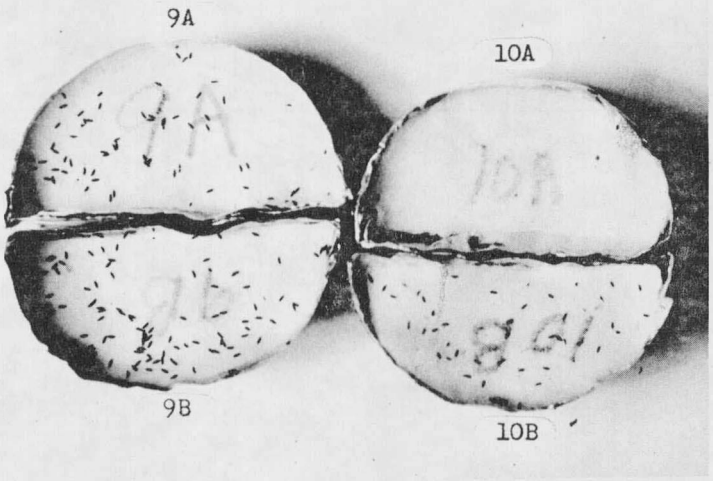



Fig. 11. Typical oviposition avoidance of a buffered pH solution.

would be necessary to postulate that there is differential resistance to toxicity between different eggs in the same batch, which can not be determined from these data.

Table X. summarizes the effect the buffered solutions had on egg viability. The material is presented as the percentage of mosquitoes in each treatment group which laid eggs in each egg number group. The "treatment groups" were the "buffered group", (experiments 1-12) and the "non-buffered group", (experiments 13-44). For example, 9.8 percent of the 77 mosquitoes involved in the buffered solution tests laid from 1-10 inviable eggs only, and so on. Looking at the totals column of this table it is seen that 29.9 percent of the 77 mosquitoes in the buffered solution tests laid only inviable eggs, while in the non-buffered tests none of 199

Table X. Comparison of egg viability as affected by type of treatment.

Expressed as percentage of mosquitoes tested in each treatment*

| | No. of Eggs Laid | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1-10 | 11-25 | 26-50 | 51-75 | 76-100 | 101-150 | 151-228 | Totals |
| Inviable Only | | | | | | | | |
| Buffered | 9.8 | 9.8 | 5.2 | 5.2 | 1.3 | 2.6 | - | 29.9 |
| Non-Buffered | - | - | - | - | - | - | - | - |
| Viable Only | | | | | | | | |
| Buffered | 3.9 | 2.6 | 3.9 | 2.6 | 1.3 | - | 1.3 | 15.6 |
| Non-Buffered | 9.0 | 1.5 | 10.0 | 5.5 | 7.5 | 12.1 | 1.5 | 47.2 |
| Both Viable & Inviable | | | | | | | | |
| Buffered | 1.3 | 1.3 | 2.6 | 7.8 | 4.2 | 5.2 | 1.3 | 24.7 |
| Non-Buffered | 0.5 | 2.0 | 1.0 | 2.5 | 2.0 | 4.0 | 4.5 | 16.6 |

* 77 females in buffered experiments
199 females in non-buffered experiments

mosquitoes involved laid only inviable eggs. Another definite effect can be seen in the percentages producing only viable eggs in the buffered and non-buffered test groups. These percentages were 15.6 percent and 47.2 percent respectively. The percentages producing both viable and inviable eggs in the two groups were 34.7 percent for the buffered group and 16.6 percent for the non-buffered group. Thus it can be seen that more inviable eggs were produced on the buffered pH than on the non-buffered solutions.

An examination of the results obtained from the distilled water vials shows the apparent random distribution of eggs, with no consistent pattern of choice of one cup over the other. This experiment with distilled water in both cups was of course designed as a control, but the random distribution noted here was characteristic of all other choices with the exception,

as has already been mentioned, of the buffered pH solutions. For this reason tables presenting the details of the other experiments have been omitted.

Certain data arrived at from the 1956 individual vial isolations and the 1957 comparison vial isolations showed comparable results. Table XI. presents this material.

Table XI. Comparison of 1956 and 1957 isolation test findings.

| Year | Percent* of Mosquitoes that: Developed No Eggs | Developed Eggs But Refused to Oviposit | Oviposited But Retained Some Eggs in Abdomen | Oviposited All Eggs | Range in Number of Eggs Produced |
|---|---|---|---|---|---|
| 1956 | 8.9 | 22.2 | 35.6 | 33.3 | 2-225 |
| 1957 | 6.9 | 27.5 | 27.9 | 22.5 | 2-228 |

* 1956 - 45 mosquitoes tested
1957 -276 mosquitoes tested

From this it can be said that with the conditions under which the tests for these two years were made, approximately one-fourth of the mosquitoes tested refused to oviposit, even though they retained fully developed eggs in their abdomens until death. This, taken with the fact that 16 (or 35.6 percent) of the 45 mosquitoes tested in 1956 and 77 (or 27.9 percent) of the 276 mosquitoes tested in 1957 laid only part of the eggs they developed strongly suggests that some oviposition stimulus was lacking. Since all of these mosquitoes were provided with a surface similar in moisture at least to that on which they oviposit in nature, it would appear evident that the presence of water alone is not a sufficient stimulus (although it

has been proven to be sufficient in certain other Aedes species) to induce complete (or even partial) oviposition in many individuals.

## FIELD AIR SAMPLING

### Methods

To obtain some data on rate of production of carbon dioxide, methane and hydrogen sulfide in the field, air samples were collected and analyzed. The technique used was modified from that used by Wallis and Wilde (1957) in their determination of carbon dioxide evolution from forest soils. For the collection of these field air samples, galvanized metal sampling funnels 6 X 8 inches were used.

A typical western wheatgrass meadow oviposition site area was chosen for the test. This field had been irrigated in a manner commonly used in the Milk River Valley: water had been turned on the field and allowed to stand for approximately thirty days. During this time a very heavy production of A. nigromaculis larvae (as well as other Aedes species) had occurred. At the end of the irrigation period, the owner had drained off as much water as possible. However, several low areas in the field contained pools of water (see Fig. 1.) which were left to evaporate, thus producing ideal oviposition sites.

For collection of samples, four locations on or near this field were chosen. One was on the mud bank of the main irrigation ditch supplying water to the field. Repeated observations had indicated that no oviposition took place in this ditch. A second location was a bare mud area

on the side of a field distribution ditch (see Fig. 2.), an area on which the 1955 hatch tests indicated that little or no oviposition took place.

The other two sample sites were areas of heavy grass-and-duff cover, one on the side of a depression, and the other on a fairly level part of the field. These were representative of areas on which the hatch tests indicated the greatest amount of oviposition occurred. In each of these four areas a sealed sampling funnel was sunk into the soil to a depth of two inches (to insure that no gas would leak out around the edges), and then left for 24 hours (Sept. 13-14) to collect whatever gases that were evolving. During the test period a maximum-minimum thermometer recorded a temperature range from 42° to 72° F. At the end of the 24 hour test period 500 ml. of air from each funnel were drawn into a gas sampling tube from which the air had just been exhausted by use of a 12 volt Trico portable vacuum pump as described by Wallis and Wilde (1957).

A control sample was collected from the air six feet above ground level. These tightly sealed sample tubes were then sent to Horvitz Research Laboratories, Houston, Texas for analysis. Due to the expense involved ($35 per sample) only this one set of samples was taken.

## Results

The analysis results received from the Horvitz Laboratories were as follows:

Table XII. Gas sample analysis.

| Sample No. | Sample Area | Percent by Volume | | | |
|---|---|---|---|---|---|
| | | Methane | Ethane* | Carbon Dioxide | Hydrogen Sulfide |
| 1 | Bare mud irrigation ditch | 0.0011 | 0.007 | 0.79 | - |
| 2 | Bare mud distribution ditch | 0.0013 | 0.0017 | 0.51 | - |
| 3 | Heavy grass cover-depression | 0.0014 | 0.0010 | 0.87 | - |
| 4 | Heavy grass cover-level area | 0.0010 | 0.0002 | 0.57 | - |
| 5 | Control-Air 6' above ground | 0.0014 | 0.006 | 0.04 | - |

* Ethane plus heavier hydrocarbons

These data show that only carbon dioxide was produced in appreciable quantities in the 24 hour test period. The surprising thing is the lack of any hydrogen sulfide in all samples. That hydrogen sulfide is produced in these fields is quite evident to the human sense of smell any time the decomposing organic material is disturbed. It can be theorized that perhaps either the sampling or analysis techniques were not capable of picking up this gas, or possibly the hydrogen sulfide does not diffuse above ground level.

## MISCELLANEOUS OBSERVATIONS

While collecting adult mosquitoes from a western wheatgrass meadow on September 10, 1957 the author happened on a large concentration of sluggish adult mosquitoes. Further observations showed that thes mosquitoes were

just emerging from an area of mud about two yards in diameter. There was a layer of pupae about three inches deep on the surface of this mud. These pupae had been deposited there when the owner had drained his field at least 36 hours earlier. Thus these pupae had survived dessication from two sunny days and were emerging in large numbers in complete absence of water. While it has long been known that mosquito pupae are capable of emerging after up to four days on moist cotton pads or filter paper in the laboratory (Morgan and Dupree-1903, and Fielding-1919), this is a case of mass emergence under field conditions.

Observations of mosquitoes feeding on the nectar of flowers growing in irrigated pastures were made on several occasions in August, 1957. Feeding was observed from about 6:30 p.m. until darkness. Coreopsis (Coreopsis atkinsonia), a composite common in these irrigated fields, was the principal species on which feeding was observed. On one occasion, both a male and a female mosquito were seen feeding from the same flower. In feeding, a mosquito would insert its mouth parts into a floret for five to ten seconds, withdraw them and immediately insert them in another floret. This procedure was sometimes interrupted by an interval in which the mosquito either remained quiet for a minute or so, or would slowly move over the flower, briefly exploring the surface with the mouthparts. The maximum time a mosquito was seen to remain on a flower was about five minutes.

## DISCUSSION

The oviposition of irrigated pasture mosquitoes is apparently not random. The results of the 1955 sod hatch tests indicate that the mosquitoes exhibit definite oviposition site choice, preferring the sloping sides of little depressions in the fields and areas with thick plant debris cover on the soil. The nature of this ability of the mosquitoes to select specific oviposition sites is not entirely understood. Several workers have demonstrated the ability of numerous species of mosquitoes to distinguish differences in moisture levels. However, soil moisture levels are evidently not the only factors involved in site choice for oviposition by the species under study in this project. The fields in this area are almost entirely under water when irrigated. Thus as the fields dry up after an irrigation all areas are wet at one time or other and yet certain areas in a field are favored over others. It would seem probable that there is some factor other than water which attracts mosquitoes to these favored spots.

The organic decomposition gases tested in this study are apparently not the attractants, as evidenced by the fact that no pattern of choice could be distinguished at any dilution of the gases tested. Neither was there any repellant action of these gases with the exception of hydrogen sulfide at a concentration greatly in excess of any amount to be found in nature.

That A. nigromaculis does have the ability to distinguish chemicals and gases is evident in the avoidance of hydrogen sulfide and of buffered

sodium phosphate-citric acid solutions. However, no data were obtained in any test conducted to indicate that any concentration of chemicals or gases likely to be found in the natural habitat would be apt to alter the behavior of this species.

From the observations of the writer it seems likely that certain physical ecological factors of the habitat are the causes of concentration of mosquitoes in the areas of heaviest oviposition. As an example, mosquitoes can be readily observed to be resting in the protection of grass stems, where the dense "tangle" of stems offers protection, while a few feet away in a bare area no mosquitoes will be found. Also, certain gross tests made with a hand aspirated hygrometer indicated that relative humidity differences may be quite large in two areas just a few feet apart.

It is possible that the combined factors of protection plus a humidity gradient may be the things that cause oviposition to be concentrated in certain locations in irrigated pastures.

## SUMMARY

The following information was gained in this study of A. nigromaculis:

1. Females appear to prefer the sloping sides of depressions in native grass pastures for oviposition sites. A thick covering of plant debris on the ground surface enhances such an area for oviposition preference.

2. Larvae are moved by the movement of irrigation water to considerable distances from their hatching site.

3. Under temperatures found in the Milk River Valley in August, a period of six and one-half to eight days is required for larval and pupal development of this mosquito.

4. Females held at a sustained temperature of 55° F. apparently will not oviposit.

5. A single female is capable of producing up to 228 eggs.

6. Hydrogen sulfide, carbon dioxide, and methane are apparently not oviposition attractants for this species even though carbon dioxide, at least, is evolved from natural oviposition sites in measurable quantities.

7. Females exhibit chemotactic sensory behavior in avoidance of certain concentrations of hydrogen sulfide gas and buffered sodium phosphate-citric acid solutions.

8. Females showed no oviposition preference for any differences in "types" of water (field, ditch, distilled and tap) tested, nor was any preference shown for any of the "texture" materials tested.

9. About 29 percent of all mosquitoes isolated in oviposition vials, in 1956 and 1957 developed eggs but refused to oviposit. Approximately 32 percent laid some eggs but refused to lay their entire clutch. Only about 28 percent deposited their entire clutch. Since all of these mosquitoes had a source of water present in the vials, it would appear that some stimulus other than mere presence of water is necessary to cause complete oviposition.

10. Under field conditions, pupae are able to continue development to adults in the absence of free water, if they remain fairly moist.

## LITERATURE CITED

Anonymous
1953 First Progress Report on Mosquito Investigations, Milk River Project, Montana. U.S.D.H.E.W., P.H.S., C.D.C., 53 pp., Salt Lake City, Utah.

Beckel, W. E.
1955 Oviposition Site Preference of Aedes Mosquitoes (Culicidae) in the Laboratory. Mosquito News 15(4):224-228.

Bodman, M. T. and N. Gannon
1950 Some Habitats of Eggs of Aedes vexans. Jour. Econ. Ent. 43(4):547-548.

Brown, A.W.A., D. S. Sarkaria, and R. P. Thompson
1951 Studies on the Responses of the Female Aedes Mosquito. Part I.- The Search for Attractant Vapours. Bull. of Ent. Res. 42:105-122.

Carpenter, S. J., and W. J. LaCasse
1955 Mosquitoes of North America. 360 pp., Univ. of Calif. Press, Berkeley, Calif.

Crumb, S. E.
1924 Odors Attractive to Ovipositing Mosquitoes (Diptera:Culicidae). Ent. News 35:242-243.

De Coursey, J. D. and A. P. Webster
1955 Effect of Insecticides and Other Substances on Oviposition by Aedes sollicitans. Jour. Econ. Ent. 45(6)1030-1034.

Dethier, V. G.
1947 Chemical Insect Attractants and Repellants. 289 pp., Blakiston Co., Phila., Pa.

Edmunds. L. R.
1955 Notes on the Biology and Seasonal Abundance of the Larval Stages of Culex tarsalis Coquillet In Irrigated Areas of Scotts Bluff County, Nebraska (Diptera:Culicidae). Mosquito News 15(3)157-160.

Fielding, J. W.
1919 Notes on the Bionomics of Stegomyia fasciata Fabr. Part I. Ann. Trop. Med. and Parasitol. 13:259-296.

Gjullin, C. M., W. W. Yates, and H. H. Stage
1950 Studies on Aedes vexans (Meig.) and Aedes sticticus (Meig.), Flood Water Mosquitoes, in the Lower Columbia River Valley. Ann. Ent. Soc. America 43:262-275.

Kennedy, J. S.
1942 On Water Finding and Oviposition by Captive Mosquitoes. Bull. Ent. Res. 32(4):279-301.

Laarman, J. J.
1955 The Host-seeking Behavior of the Malaria Mosquito Anopheles maculipennis atroparvus. Acta Leidensia 25:1-144. University of Leiden, Netherlands.

Mail, G. A.
1934 The Mosquitoes of Montana. Mont. St. Coll. Bull. No. 288, 72 pp.

Mayne, B.
1926 Notes on the Influence of Temperature and Humidity on Oviposition and Early Life of Anopheles. Publ. Health Rpts. 41:986-990.

Morgan, H. A. and J. W. Dupree
1903 Development and Hibernation of Mosquitoes. Bull. U. S. Bureau Ent. 40:88-92.

Parker, A. H.
1948 Stimuli Involved in the Attraction of Aedes aegypti, L., to Man. Bull. Ent. Res. 39:387-397.

1952 The Effect of a Difference in Temperature and Humidity on Certain Reactions of Female Aedes aegypti (L.). Bull. Ent. Res. 43:221-229.

Reeves, W. C.
1951 Field Studies on Carbon Dioxide as a Possible Host Stimulant to Mosquitoes. Proc. Soc. Exp. Biol. Med. 77(1):64-66.

1953 Quantitative Field Studies on a Carbon Dioxide Chemotropism of Mosquitoes. Jour. Trop. Med. (Hyg) 2(2):325-331.

Rudolfs, W.
1922 Chemotropism of Mosquitoes. N. J. Ag. Exp. Sta. Bull. 367, 23 pp.

Sharma, H. N. and S. K. Sen
1921 Oviposition in Culicidae. Rept. Proc. 4th Entomological Meeting, Pusa, India, pp. 192-204.

Thomson, R. C. M.
1938 The Reactions of Mosquitoes to Temperature and Humidity. Bull. Ent. Res. 29:125-139.

Trembley, H. L.
1955 Mosquito Culture Techniques and Experimental Procedures. Amer. Mosquito Control Assoc. Bull. No. 3, 73 pp.

Wallis, R. C.
1954A A Study of Oviposition Activity of Mosquitoes. Amer. Jour. Hyg. 60(2):135-168.

1954B Observations on Oviposition of Two Aedes Mosquitoes (Diptera Culicidae). Ann. Ent. Soc. America 47(3):393-396.

1954C The Effect of Population Density and of NaCl Concentrations in Test Series in Laboratory Experiments with Ovipositing Aedes aegypti. Mosquito News 14(4):200-204.

1955 A Study of the Oviposition Activity of Three Species of Anopheles in the Laboratory. Amer. Jour. Trop. Med. and Hyg. 4(3):557-563.

Wallis, G. W. and S. A. Wilde
1957 Rapid Method for the Determination of Carbon Dioxide Evolved from Forest Soils. Ecology 38(2):359-361.

Willis, E. R.
1947 The Olfactory Responses of Female Mosquitoes. Jour. Econ. Ent. 40(6)769-778.

Willis, E. R. and L. M. Roth
1952 Reactions of Aedes aegypti to Carbon Dioxide. Jour. Exptl. Zool. 121(1):149-179.

Woke, P. A.
1955 Deferred Oviposition in Aedes aegypti (Linnaeus) (Diptera: Culicidae). Ann. Ent. Soc. America 48:39-46.