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.

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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.

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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

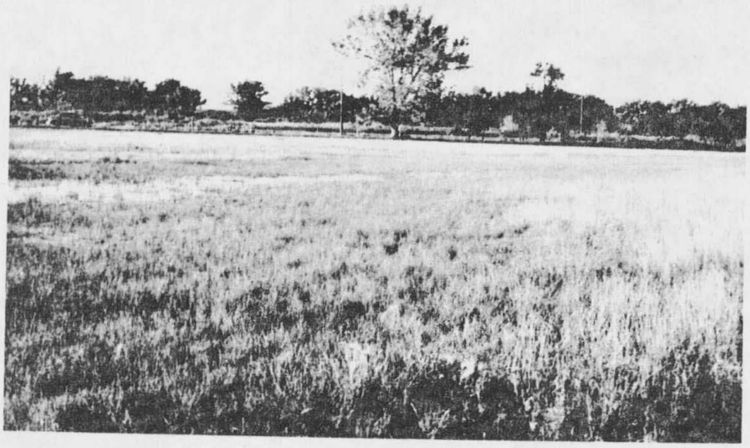

Fig. 1. Typical western wheatgrass pasture showing residual surface water ideal for mosquito production.

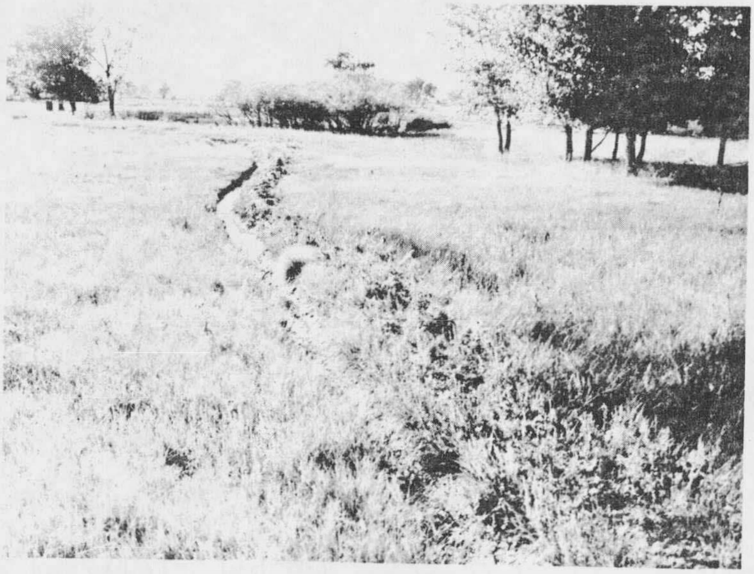

Fig. 2. A contour ditch irrigation system.

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.

Set No.	Depression Sides: No. of Samples Taken	Depression Sides: No. of Samples W/Hatch	Depression Bottoms: No. of Samples Taken	Depression Bottoms: No. of Samples W/Hatch	Level Areas: No. of Samples Taken	Level Areas: 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* No. of Samples Taken	Very Heavy Duff Cover* No. of Samples W/Hatch	Heavy Duff Cover No. of Samples Taken	Heavy Duff Cover No. of Samples W/Hatch	Moderate Duff Cover No. of Samples Taken	Moderate Duff Cover No. of Samples W/Hatch	Light Duff Cover No. of Samples Taken	Light Duff Cover 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