STIMULI AFFECTING SELECTION OF OVIPOSITION SITES BY *AEDES VEXANS* (DIPTERA: CULICIDAE): MOISTURE[1]

DANIEL STRICKMAN[2]

Department of Entomology, University of Illinois, Urbana, Illinois 61801

ABSTRACT. Experiments were done to examine the effect of moisture in the soil on selection of oviposition sites by *Aedes vexans*. In the laboratory, a device was designed and constructed which presented gravid mosquitoes with a choice of surfaces held at constant moistness. Using this device it was demonstrated that damp surfaces were preferred to either very wet or dry ones. In the field, addition of water to an oviposition site when the area as a whole was dry was sufficient to attract ovipositing mosquitoes. Oviposition was enhanced when suitable levels of moisture in the field were maintained by (1) elevation in relation to water table, (2) by kind and amount of detritus on the soil, (3) by nature and density of canopy, and (4) by frequency and abundance of rainfall.

The selection of suitable oviposition sites by *Aedes* (*Aedimorphus*) *vexans* (Meigen) affects its survival greatly. Females place their eggs directly on the soil at sites which are (1) likely to retain sufficient moisture for successful embryonation and (2) likely to be inundated at some future date. Ovipositing females manage to select suitable sites by means of cues based on a number of environmental factors. The stimulus under consideration in this paper is moisture in the soil. The observation that concentrations of eggs are often found at particular levels along a slope has led to the proposal that the horizon with many eggs maintained a suitable moisture level for a longer period than did surrounding elevations (Horsfall 1963, Horsfall et al. 1973, Horsfall et al. 1975, Novak 1976). Another moisture-related correlative of locations with large numbers of eggs is dense cover over the soil (Bodman and Gannon 1950, Horsfall et al. 1973). Detritus, piles of twigs, and low herbal canopy are usually associated with the greatest numbers of eggs within an oviposition site (Horsfall et al. 1973).

The use of tests of moisture to predict oviposition by flood-water mosquitoes has attracted the attention of previous workers. Among these, Horsfall et al. (1975) reported that most eggs of *Ae. vexans* at a flood-plain site were found where soil was "moist but not glistening." In contrast, waterlogged areas where the soil was "glistening" contained no eggs. Another method of testing moisture in the soil was described by Olson and Meek (1977) in a study of oviposition by *Psorophora* (*Grabhamia*) *columbiae* (Dyar and Knab) in the laboratory and in rice fields. Using a technique involving manipulation and observation of soil ("hand squeeze soil ball technique"), they were able to show that most eggs were deposited on soil within certain limits of moistness.

Some of the experiments performed for the current study were designed to demonstrate the importance of moisture to ovipositing *Ae. vexans*. Experiments in the laboratory with caged mosquitoes were undertaken at the University of Illinois in Urbana. Experiments in the field with natural populations were performed with the help of the Macon Mosquito Abatement District in Decatur, Illinois. Further fieldwork was done in Decatur in order to examine the relationships be-

[1] This work was done as a part of a doctoral thesis completed in 1978 at the University of Illinois. The author wishes to express his thanks to Dr. William R. Horsfall for advice and to the Macon Mosquito Abatement District for financial and material support.

[2] Current address: Instituto de Ciencias Basicas, Universidad Nacional de Asuncion, Casilla de Correo 1039, Asuncion, Paraguay. Please address reprint requests c/o Department of Entomology, University of Illinois, Urbana, Illinois 61801.

tween soil moisture, several environmental features, and oviposition. The object of this work was to provide information which would help in the prediction of the location of large concentrations of larvae following major rains.

MATERIALS AND METHODS

LABORATORY: In order to provide gravid *Ae. vexans* in the laboratory with a choice of surfaces varying in moistness, an apparatus herein referred to as the COMOLA (COnstant MOisture Level Apparatus) was designed and built (Figure 1). The COMOLA consisted of 3 main parts: Cage, deck, and surface assemblies. The square cage (51 cm high x 91 x 91 cm) was constructed from 5-cm thick styrofoam sides (a) and a nylon tulle top (b). Access was provided by a knitted sleeve on each side of the cage. The cage rested on a horizontal deck (c), comprised of a 91 x 91 cm piece of 6-mm thick plywood re-enforced along the undersides of its edges by 1.9 x 4.4 cm strips of wood (d). The deck was penetrated by 12 holes (e) (each 12 cm in diameter) arranged in a circular pattern. The entire deck was coated with a polyurethane finish and its top surface was painted flatblack. During experiments a flask containing a cheesecloth wick and honey-water solution (ca. 10%) rested on the center of the deck. Each of the 12 surface assemblies included a section of plexiglass pipe (f) (inside diameter of 10.8 cm, height of 15.2 cm, wall-thickness of 3 mm) filled with sand (g) (natural grain, silica sand, mean diameter ± standard deviation for 100 grains equal to 0.46 ± 0.16 mm). These upright columns of sand rested in cut-down, plastic, 1-gal jars (h), arranged so that the circular surfaces of sand were flush with the top of the deck. The deck was supported at its corners by bricks to accommodate the surface assemblies. A piece of surgical tubing (i) provided a connection between the plastic jars and the elevated trays which served as reservoirs (j).

To use the COMOLA, each reservoir was adjusted to ca. the desired height and then filled with water. Next, the piece of surgical tubing was filled, forming a siphon between the reservoir and the plastic jar. Water then flowed into the plastic jar and was soaked up by the sand.

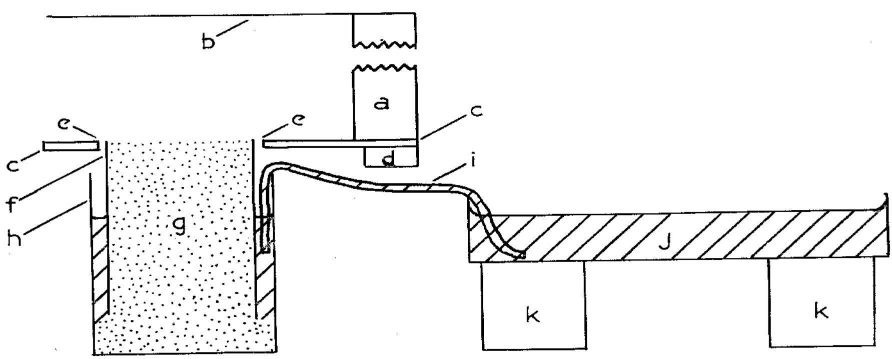

Figure 1. Cross-sectional diagram of a portion of the COMOLA, a device designed to present oviposition surfaces of constant moistness to flood-water mosquitoes in the laboratory. Cage: a. Styrofoam side, b. tulle top. Deck: c. Plywood deck, d. re-enforcing strip of wood, e. edges of hole in deck. Surface assembly: f. Section of plexiglass pipe, g. sand, h. cut-down jar, i. water-filled surgical tubing, j. reservoir, k. supports for reservoir.

Additions of water to the reservoir were made until the desired level in the plastic jar was attained. Plastic food wrap was placed over the reservoir to retard evaporation. Though the oviposition surface and the plastic jar remained exposed to evaporation, the desired level in the jar was maintained by flow through the siphon connected to the reservoir. Daily addition of water to each reservoir made it possible to keep the artificial water-table level (i.e. the distance between the level in the jar and the surface of the sand) continually within 1 mm of the desired value.

Mosquitoes were reared for use in the COMOLA from eggs collected from the field in Macon Co., Illinois. Eggs were hatched and larvae reared according to the methods described in detail by Horsfall et al. (1973). A few days following emergence, the unmated females were offered daily bloodmeals on a human for 4 consecutive days. On the 4th day of bloodmeals the mosquitoes were placed in the COMOLA.

The experiment with different moisture levels was conducted with 83 gravid *Ae. vexans*. These were exposed to the surfaces in the COMOLA during 7 days under constant light. The COMOLA was arranged with artificial water tables at levels of 13, 20, 40, 60, 80, and 100 mm below the exposed surfaces of sand. Each water-table level was maintained under 2 surfaces located on opposite sides of the deck. At the conclusion of the experiment, the top centimeter of sand was removed from each surface, the eggs floated up from the sand in saturated salt solution, and the eggs counted. For purposes of analysis, the 4 driest surfaces were grouped together as "dry," the 4 wettest surfaces were grouped together as "wet," and the remaining 4 surfaces were grouped together as "damp."

FIELD. A number of experiments were conducted in the field to demonstrate the importance of moisture in attracting ovipositing females, and to examine the relationships between aspects of the environment, soil moisture, and oviposition. Most of these experiments involved the separation of eggs from the soil. To do this, a technique described in detail by Horsfall (1956) was used. Briefly, this technique consisted of removing a sample of the upper 2 cm of soil from an oviposition site with a sharpened masonry trowel. The sample was taken to the laboratory and washed through a series of successively finer screens. Ultimately, detritus and the eggs themselves were caught on a 1/100 mesh screen. This mixture of material was then separated further using the tendency of the eggs to float in saturated salt (sodium chloride) solution and to sink in tap water. As a final step, the eggs were mechanically pipetted from the remaining detritus while under observation with a dissecting microscope. Once eggs were obtained they were identified to species by viewing their shape and chorionic patterns at 70 to 100 magnifications against a brightly-lit, dull-black background (Horsfall and Craig, 1956). Determination of the age of eggs was accomplished by breaking each egg with a curved needle and observing its contents. When the embryo streamed out as a thick, milky fluid and the shell broke irregularly, the egg was considered to be "new" (i.e. less than 24 hours since its deposition). If the embryo had an eyespot or signs of segmentation, the egg was considered to be "residual" (i.e. greater than 24 hours since its deposition). Observation in the laboratory of *Ae. vexans* eggs of known age established that the interpretations of age were correct.

Two experiments were performed to examine the attraction of ovipositing females in the field to artificially moistened soil. The location of these experiments was an old, shallow trench about 25 m long, 5 m wide, and up to 1.5 m deep. The site was surrounded by cottonwood (*Populus deltoides*) and willow (*Salix* sp.) trees. The experiments were conducted during a period in July, 1977, when the area as a whole was dry in order to get maximum contrast between artificially moistened and natural, dry soil. Water was applied using a siphon leading from an 11-liter bucket. The siphon was made from vinyl tubing (6-mm diameter) blocked at its outflowing end with a piece of wooden dowel. The dowel was grooved to allow a slow flow of water. The rate of flow was adjusted to a steady drip using a hose clamp around the section of tubing which contained the dowel.

In the first experiment, a wick of urethane foam (1.9 x 5 cm in cross section) buried in the bottom of the trench was kept continually wet during one night. The following morning, samples of soil in concentric circles were removed from around the wick.

In the second experiment, water was dripped directly into a slot cut into the sloping side of the trench. The slot was 2.5 cm deep and 15.2 cm long, with its long dimension perpendicular to the direction of slope of the side of the trench. As the water seeped downhill, a gradient of moisture was established with the wettest soil nearest the slot. After 3 nights of exposure, the area was sampled by removing adjacent squares of soil (15 x 15 cm) in a row from the slot running down the slope of the depression.

In this study, Bouyoucos® blocks and a Bouyoucos meter (Beckman Instruments) were used to measure soil-water potential (see below). The blocks (1.5 x 3 x 4 cm) were made of gypsum and contained 2 flat electrodes. A block was installed by fitting it snugly into a hole in the surface of the soil. It was then covered with the same detritus layer present in the surrounding area. When moisture in the soil increased, more water penetrated the block, lowering the electrical resistance between the 2 electrodes. The Bouyoucos meter, a modified ohm-meter, was used to measure this change. It was calibrated in percentage of available moisture, a scale related to plant response. One-hundred percent available moisture corresponded to total saturation of the soil while 0% available moisture corresponded to the permanent wilting point (Hagan et al. 1959).

The effect of elevation on soil moisture was studied during the summer of 1977 in the same area as the experiments with artificial addition of water to the ground. Bouyoucos blocks were installed at 8.9, 14.0, and 33.0 cm above the bottom of the depression. A 4th Bouyoucos block was installed about 100 m away in a shallow oviposition site shaded by a single cottonwood tree. The elevation of this upper block was about 100 cm above the bottom of the trench. Readings from the Bouyoucos blocks and a rain gauge were taken daily.

Two sites with different kinds of cover over the ground were used in a comparison of soil-moisture during the summer of 1976. One site was covered with a thick layer of detritus from the willow trees which formed a dense, low canopy over the area. The other site, 1 km away, had no arboreal canopy but was covered by a dense growth of smartweed (*Polygonum* sp.), a broad-leaf, erect herb. A Bouyoucos block was installed at each site and readings were taken daily. Rainfall records were taken from the National Oceanic and Atmospheric Administration (1976).

The relationship between changes in soil moisture brought about by rainfall and deposition of eggs by *Ae. vexans* was studied at the trench where Bouyoucos blocks were installed in 1977. Eggs were extracted from 3 or 4 squares of soil (15 x 15 cm) taken each day from the side of the trench. The samples were taken in adjacent strips running down the slope of the depression. An effort was made to avoid disturbing the areas that had not been sampled. The squares sampled a range of elevations each day and, as data was gathered, the middle elevation for sampling was selected from the level with the greatest concentration of eggs on previous days. The daily means of the readings of the 3 Bouyoucos blocks in the trench were used in comparisons of soil moisture and deposition of eggs.

RESULTS AND DISCUSSION

The direct influence of surface moisture on the choice of oviposition sites by *Ae. vexans* was demonstrated in the laboratory using the COMOLA (Table 1). Gravid mosquitoes given simultaneous access to 12 surfaces of sand which differed in moisture tension deposited the majority of their eggs on surfaces which were damp but not waterlogged. Mosquitoes were able to distinguish between surfaces dampened by artificial water tables which differed by only 20 mm. Despite the indicated preference for a limited range of moisture tensions, at least some eggs were deposited on each of the 12 surfaces in the experiment.

Under field conditions, addition of moisture to soil in a known oviposition site was sufficient to attract female *Ae. vexans* to deposit eggs. A total of 175 eggs was found in soil collected around a urethane-foam wick which had been moistened during a single night. Of these eggs, 170 had been deposited during the previous 24 hr. One-hundred sixty-nine of these new eggs were recovered from soil within 10 cm of the wick. The 5 residual eggs indicated that, prior to treatment, the site was of low suitability for oviposition. The addition of water had made the location highly attractive to gravid mosquitoes.

Since moisture in the soil is one of the stimuli involved in selection of oviposition sites, it follows that much may be learned about distribution of eggs by studying the distribution of moisture in the soil. In the course of this study the following 3 qualities of a site were examined in relation to soil moisture: Elevation of a site relative to its surroundings, cover both on and above a site, and the amount and time of rainfall. In these studies, soil-water potential was used as a measure of moistness. Soil-water potential is one of the 2 qualities of water in the soil which may be expressed quantitatively. It is defined as the amount of work necessary to transport water out of the soil. This measure of moisture is quite different from simple water content, or the mass of water in the soil divided by the mass of dry soil (Soil Physics Terminology Committee 1963). The relationship between water content and soil-water potential cannot be described by simple equations. What is more, this relationship varies for different soils and within the same soil under different conditions. Although, in theory, water content is very simple to measure, oxidation of organic matter during drying of the soil, vagaries of laboratory ovens, and the lack of reproducibility of the "dry" state may cause major errors. Soil-water potential, on the other hand, is easily measured using tensiometers or Bouyoucos blocks. Also, it is more closely related to plant response and to what we perceive as "wetness" (Gardner 1965, Haise and Hagan 1967).

As suggested by previous workers (Horsfall 1963, Horsfall et al. 1973, Horsfall et al. 1975, Novak 1976), small changes in elevation along a slope may make a great difference in the effect of rainfall on soil moisture and, therefore, the distribution of eggs. This was demonstrated during a summer using Bouyoucos blocks installed in the surface of the soil at 2 oviposition sites. In one site, percentage of available moisture was

Table 1. Distribution of eggs deposited by 83 gravid females of *Aedes vexans* given simultaneous access to surfaces held at various moisture levels. Urbana, Illinois, 1977.

Level of moisture⁺	No. of surfaces	No. of eggs of *Aedes vexans* per surface		
		Maximum	Minimum	Mean⁺⁺ ± Standard deviation
Dry	4	36	4	$22^a \pm 14$
Damp	4	382	103	$254^b \pm 135$
Wet	4	130	27	$64^a \pm 46$

⁺ Dry: Surfaces 80 and 100 mm above water level. Damp: Surfaces 40 and 60 mm above water level. Wet: Surfaces 13 and 20 mm above water level.

⁺⁺ Means not sharing a common superscripted letter were significantly different at the 95% level.

very similar at a reference level (where most eggs were deposited) and at an elevation near the lowest portion of the site, 5.1 cm below the reference level (Figure 2a). Farther up the slope, at 19 cm above the reference level, the soil was always slightly drier than at the elevation of maximum oviposition, except during periods when too little water remained in the soil to register on the Bouyoucos meter (Figure 2b). Eighty-six centimeters above the reference level, in a nearby depression containing few eggs, percentages of available moisture varied greatly, going up to 100% following rains but returning to near dryness in only 3 or 4 days (Figure 2c).

Further evidence that the concentration of eggs at certain elevations is caused by an attraction of gravid females to suitable moisture levels in the soil was provided by another experiment in the field (Table 2). During 3 nights when the area as a whole was relatively dry, water was dripped onto the slope of an oviposition site. Recently deposited eggs of *Ae. vexans* were concentrated near the source of water at the highest elevation sampled. Residual eggs deposited prior to the period of moistening were concentrated farther down the slope where, under previous, natural conditions, the soil had remained moist during longer periods.

Indications of the importance of cover over the ground to soil moisture are evident in a comparison of the Bouyoucos-

Table 2. Position of eggs deposited by *Aedes vexans* on a slope prior to and during moistening. Water was dripped onto the slope 60 cm above the lowest point sampled during 3 nights. Decatur, Illinois, July 13–16, 1977.

Distance along surface above lowest point sampled (cm)	No. of eggs deposited	
	Prior to moistening (residual eggs)	During moistening (new eggs)
45–60	73	218
30–45	152	44
15–30	348	10
0–15	45	0

block readings from thickly and sparsely covered sites monitored in 1976 (Figure 3). Canopy and thick detritus tended to retard the drying of soil relative to the more sparsely covered site following rain. This effect on moisture probably accounts for the greater numbers of eggs of *Ae. vexans* found by Horsfall et al. (1973) and by Bodman and Gannon (1950) in shaded and covered situations. The thicker protective layer also prevented penetration of moisture to the soil when a long, dry period had desiccated the detritus layer itself (see June 18 and 19, Figure 3).

Once water in an oviposition site has dissipated following flooding, rainfall determines the soil moisture of the area as a whole. Figures 2 and 3 clearly illustrate that the major trends in soil moisture throughout a summer are determined by the time and amount of precipitation. The effect on oviposition of the changes in soil moisture brought about by rainfall are illustrated in Figure 4. Although there was a period when mosquitoes did not oviposit significantly on moist soil (July 31-August 6) and a period when some eggs were deposited on dry soil (July 4–8), the majority (84%) of eggs were deposited when percentages of available moisture were greater than 75%.

The distribution of moisture in the soil and its effect on oviposition by *Ae. vexans* contribute to an interesting and important part of the focus of this species. Once located in the vicinity of an oviposition site, gravid females avoid dry soil and seek moist soil for deposition of eggs. Since the distribution of moist soil is influenced by the previous pattern of rainfall during that particular season, by the location of detritus and shade, and by small irregularities in the terrain, these features of the environment have a great effect on the actions of the females at the oviposition site.

Knowledge of the role of soil moisture in the focus of *Ae. vexans* may be used to advantage by mosquito abatement districts charged with managing this species.

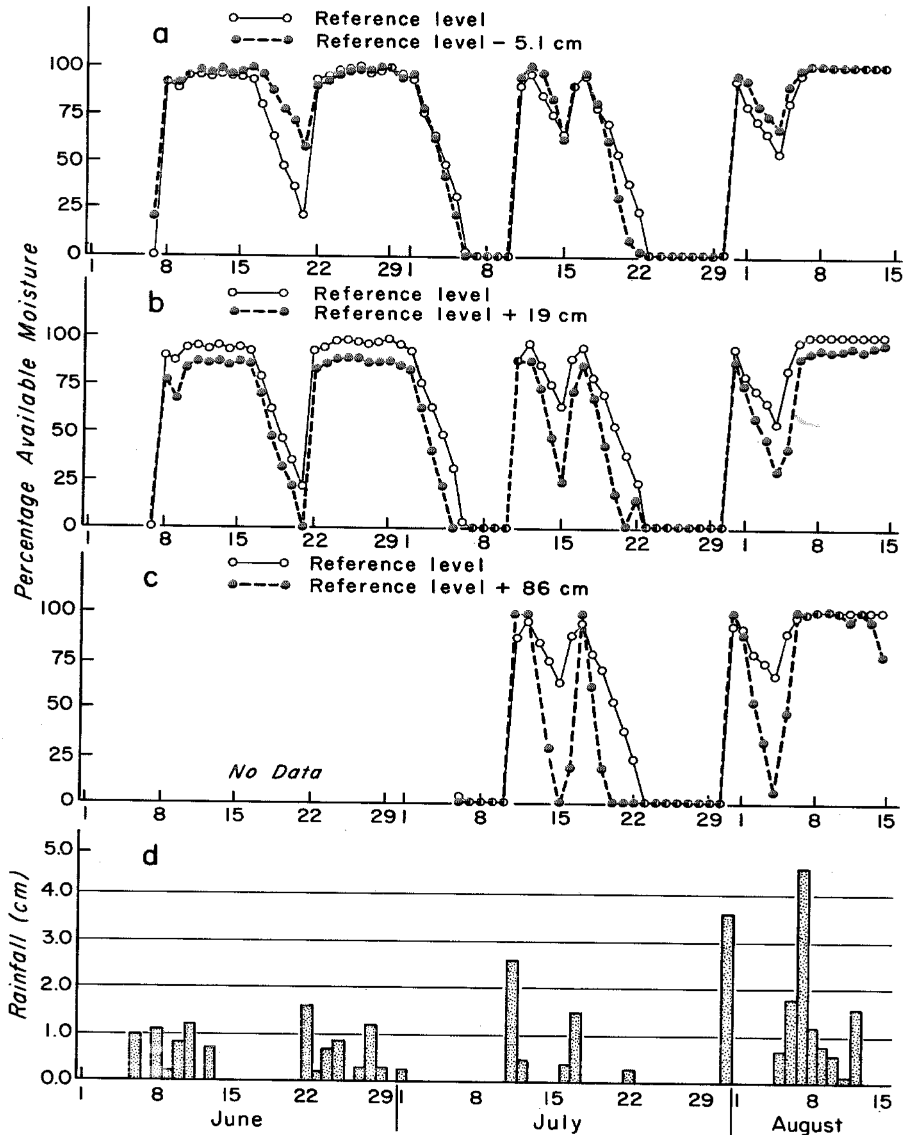

Figure 2. Comparison of percentage available moisture in the superficial layer of the soil of an upland site at a reference level and at (a) 5.1 cm below the reference level, (b) 19 cm above the reference level, and (c) approximately 86 cm above the reference level. Rainfall (d) is also indicated. Decatur, Illinois, 1977.

Following heavy rains that flood more sites than the district can treat, an examination of previous rainfall records and of characteristics of individual sites may aid in determining which locations are likely to produce the most larvae. If, during the last opportunity females had to oviposit, precipitation was scarce or fell over a short period of time, then the drier sites (i.e. more elevated, less detritus and canopy) would probably contain few eggs. If, on the other hand, rain fell during a number of days while gravid females were present, even the driest sites would probably have been moist enough to receive a large proportion of the eggs deposited. Bouyoucos blocks could be used to refine this method further. Readings from Bouyoucos blocks at representative sites throughout a district would provide precise information on moisture conditions in the field. When flooding rains did come, the manager of the district would then know what kinds of sites were likely

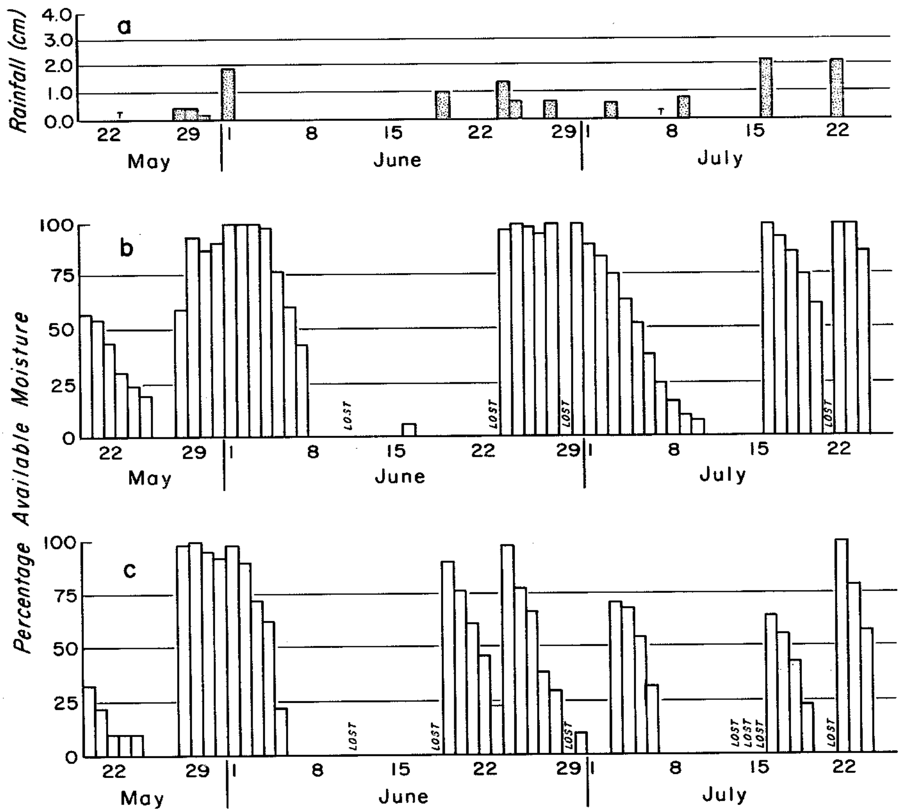

Figure 3. Percentage available moisture in the surface of the soil at a thickly covered site (b) and at a nearby, sparsely covered site (c). Rainfall (a) for the area (National Oceanic and Atmospheric Administration, 1976) is also indicated. Decatur, Illinois, 1976.

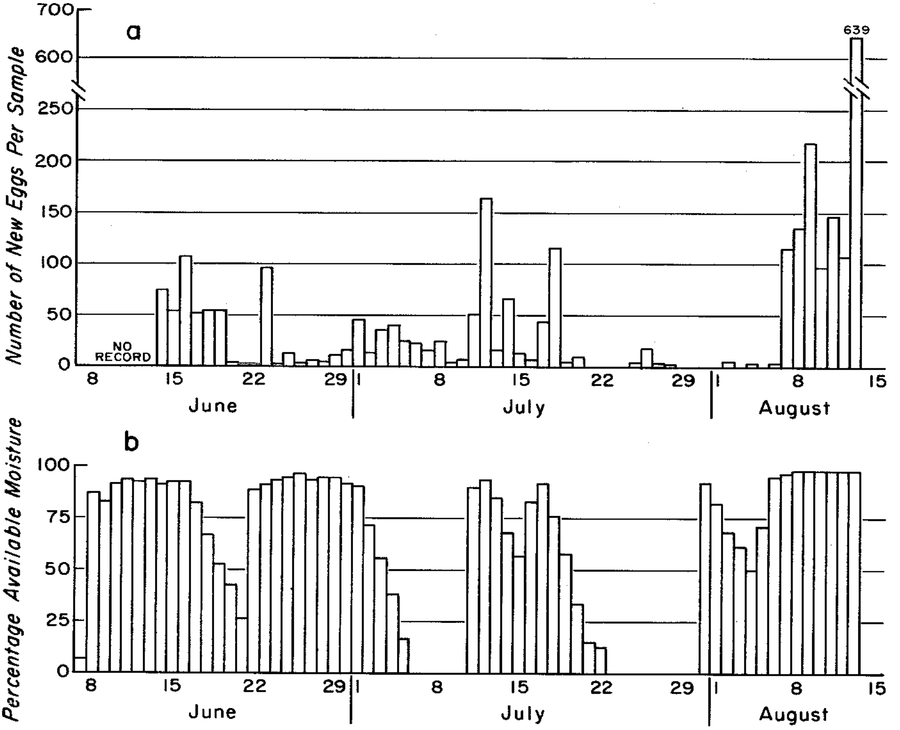

Figure 4. (a) Daily number of new eggs of *Aedes vexans* removed per sample of soil taken from a trench-like oviposition site. (b) Daily mean of readings of 3 Bouyoucos blocks installed in the surface of the soil at the oviposition site. Decatur, Illinois, 1977.

to contain the most eggs. Using this information, the manager could deploy his resources of personnel and equipment in a more efficient manner.

References Cited

Bodman, M. T. and N. Gannon. 1950. Some habitats of eggs of *Aedes vexans*. J. Econ. Entomol. 43:547–548.

Gardner, W. H. 1965. Water content. In: Methods of Soil Analysis Part 1, C. A. Black, ed., American Society of Agronomy, Inc., Madison, Wisc., U.S.A., pp. 82–127.

Hagan, R. M., Y. Vaadia and M. B. Russell. 1959. Interpretation of plant responses to soil moisture regimes. Advances in Agron 11:77–98.

Haise, H. R. and R. M. Hagan. 1967. Soil, plant, and evaporative measurements as criteria for scheduling irrigation. In: Irrigation of Agricultural Lands, R. M. Hagan, H. R. Haise, and T. W. Edminster, eds., American Society of Agronomy, Inc., Madison, Wisc., U.S.A., pp. 577–604.

Horsfall, W. R. 1956. A method for making a survey of floodwater mosquitoes. Mosquito News 16:66–71.

Horsfall, W. R. 1963. Eggs of floodwater mosquitoes (Diptera: Culicidae) IX. Local distribution. Ann. Entomol. Soc. Amer. 56:426–441.

Horsfall, W. R. and G. B. Craig, Jr. 1956. Eggs of floodwater mosquitoes IV. Species of *Aedes* common in Illinois (Diptera: Culicidae). Ann. Entomol. Soc. Amer. 49:368–374.

Horsfall, W. R., R. J. Novak and F. L. Johnson. 1975. *Aedes vexans* as a flood-plain mosquito. Environ. Entomol. 4:675–681.

Horsfall, W. R., H. W. Fowler, Jr., L. J. Moretti and J. R. Larsen. 1973. Bionomics and embryology of the inland floodwater mosquito *Aedes vexans*. University of Illinois Press, Urbana, Ill., U.S.A., 212 pp.

National Oceanic and Atmospheric Administration. 1976. Climatological Data: Illinois. Vol. 81, nos. 5–7.

Novak, R. J. 1976. The influence of oviposition site on focality of the inland floodwater mosquito. Ph.D. thesis, Department of Entomology, University of Illinois, Urbana, Ill., U.S.A., xi + 176 pp.

Olson, J. K. and C. L. Meek. 1977. Soil moisture conditions that are most attractive to ovipositing females of *Psorophora columbiae* in Texas ricelands. Mosquito News 37:19–26.

Soil Physics Terminology Committee. 1963. Report (corrected text). Bull. Internat. Soc. Soil Sci. 23:7–10.

VALIDITY OF LARVAL SURVEYS TO ESTIMATE TRENDS OF ADULT POPULATIONS OF *ANOPHELES ALBIMANUS*

DONALD L. BAILEY, RONALD E. LOWE, P. E. KAISER, DAVID A. DAME AND JOYCE E. F. FOWLER

Insects Affecting Man and Animals Research Laboratory, Agricultural Research, Science and Education Administration, USDA, Gainesville, Florida 32604

ABSTRACT. Larvae of *Anopheles albimanus* Wiedemann were collected from representative breeding sites from July 18, 1976, to March 25, 1978 in conjunction with a sterile insect release program on the Pacific coast of El Salvador, Central America. The number of larvae collected per 100 dips from breeding sites was compared with the number of adults captured per man-hour at specified cattle stables throughout the area during the same period. It was found that the larval surveys were not an accurate indicator of the actual population during the rainy season. The study shows a need for a more reliable system of sampling *An. albimanus* larvae in wet season flooded areas if larval collections are used to show relative abundance of that species.

INTRODUCTION

The sterile insect technique (SIT) has shown various degrees of success since it was first proposed by Knipling (1955). Some insects have been successfully controlled or eradicated from certain geographical areas using SIT, while other control efforts have been unsuccessful. One successful program reported by Lofgren et al. (1974) virtually eliminated a population of *Anopheles albimanus* Wiedemann from the area around Lake Apastepeque in El Salvador, Central America. An important reason for the success of that project was the fact that the test site chosen was ideal for the use of a SIT (Breeland et al. 1974). It consisted of a volcanic lake and a nearby rainy-season lagoon, both of which were isolated from other *An. albimanus* breeding by several kilometers. The lake supported larval populations only in a narrow band of vegetation around the periphery. Because the total breeding area was well defined, it was possible to place released sterile males in close proximity to the known location of emerging females for an optimum mating potential.