STIMULI AFFECTING SELECTION OF OVIPOSITION SITES BY *AEDES VEXANS*: CONDITIONING OF THE SOIL[1]

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ABSTRACT. Three experiments were performed to determine whether previous history, or conditioning, of soil influences the final selection of oviposition sites by *Aedes vexans*. Two of these experiments involved offering gravid mosquitoes a choice of kinds of soil on which to oviposit. Caged mosquitoes in the laboratory and a natural population of mosquitoes in the field preferred to oviposit on artificially moistened soil from oviposition sites rather than on artificially moistened soil from sites that had had no history of flooding. Under natural conditions, a population ceased to oviposit when a major flood covered elevations of the usual oviposition sites. When the water receded, oviposition commenced again. Selection of oviposition sites that are suitably moist and which have been favorably conditioned probably help assure that eggs are located in areas that are likely to flood.

The locations of eggs of *Aedes (Aedimorphus) vexans* (Meigen) are determined by the movements of the gravid females. First, females arrive in the general vicinity of the oviposition sites, aggregating near upland depressions, woodland pools, or flood plains (Horsfall 1963). Next, the mosquitoes select appropriate surfaces on which to oviposit. One of the stimuli which affects ovipositing mosquitoes during the final stage of selection is moistness of the soil (Horsfall 1963, Horsfall et al. 1973, Horsfall et al. 1975, Novak 1976, Strickman 1980). In addition, the existence of a stimulus created by the prior history or conditioning of the soil is suggested by the observation that eggs are generally found in sites subject to flooding even though rain often makes other kinds of sites suitably moist.

The experiments reported in this paper were designed to determine whether conditioning of soil has an effect on selection of oviposition sites by *Ae. vexans*. Tests in the field and laboratory compared the number of eggs deposited on soil from oviposition sites and on soil from other locations. These experiments were performed in central Illinois in 1977.

MATERIALS AND METHODS

Three experiments were performed to determine whether conditioning of soil influenced selection of locations for oviposition by *Ae. vexans*. One experiment was a comparison in the laboratory between soil from an oviposition site and soil from an unflooded hillside. Soil was collected in Urbana, Illinois, by driving plexiglass rings (6.4 cm in diameter, 1.9 cm high) into the ground and then cutting them free together with their cylinders of soil. In the laboratory each cylinder of soil was placed on a pad of urethane foam (2.5 cm thick) without removing the plexiglass ring. The pad rested in a small pan (8 × 12 cm) with water deep enough to come within 2 cm of the top of the cylinder. Soil from the oviposition site was mucky and foul from a recent flooding. The soil from the hillside was dry when collected and had little detritus on the surface.

Three cylinders of each kind of soil were used in the experiment. The 6 cylinders were arranged in a circular pattern in which each type of soil alternated. In the middle of the circle was a flask of

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10% honey solution containing a cheesecloth wick. The samples of soil and flask of honey solution were covered with a cage (91 × 91 cm × 51 cm high) made of styrofoam sides and a nylon tulle top. Females of *Ae. vexans* were reared from eggs according to methods described by Horsfall et al. (1973) and then offered bloodmeals on a human for 4 consecutive days before the experiment. Forty-eight of these gravid, unmated mosquitoes were exposed to the samples for 7 days at 25°C under a regimen of 16 hours of daily light. Water levels were adjusted twice per day to compensate for evaporation. At the conclusion of the experiment, eggs were extracted from the samples of soil using the method of screening, flotation in saturated salt solution, and pipetting described by Horsfall (1956). The number of eggs of *Ae. vexans* in each sample was then counted.

In a second experiment, samples of different soils were artificially moistened and presented to a natural population of *Ae. vexans* in the field. The site was an old trench in Decatur, Illinois. It was about 1.5 m deep and shaded by cottonwood (Salicaceae: *Populus deltoides*) and willow (Salicaceae: *Salix* sp.) trees. Each sample of soil (15 × 15 cm × 2.5 cm thick) rested on a water-soaked pad of urethane foam (2.5 cm thick). A plastic-lined depression was dug beneath each pad so that the surface of the sample was flush with the surrounding ground. The moisture in a sample was maintained by the constant drip of a siphon leading from an 11-liter bucket (Strickman, 1980). Four samples were exposed to the resident natural population of mosquitoes during each of 2 nights in July, 1977. Two of the squares of soil were taken from a hillside which never flooded. These were covered by a natural layer of dead vegetation and emergent, living grass 8 cm high. The other 2 squares were from the trench itself, a known oviposition site. These samples had a superficial layer (1 cm thick) of detritus from willows. The 4 squares of soil were arranged in a row along the bottom of the trench with 35 cm between each square. After a single night of exposure, the samples were taken to the laboratory and refrigerated. Within 24 hr eggs were extracted from the samples of soil. Eggs of *Ae. vexans* were grouped according to age. This was done by breaking each egg and observing the contents (Strickman 1980). An egg designated "new" was less than 24 hr old at the time of collection.

The third experiment involved extraction of eggs from an unaltered site before, during, and after a flood in May, 1977. The site consisted of the eastern slope of an old dike on the flood plain of the Sangamon River in Piatt County, Illinois. The dike was about 3 m high and, like the rest of the area, well shaded by an arboreal canopy. Rains in April had produced a brood of *Ae. vexans* which had not dispersed significantly from the area due to cool nocturnal temperatures. Between 3 and 10 samples of soil (15 × 15 cm × 2 cm thick) were collected on most days of the observation period beginning 2 days before major flooding commenced. When no water was present above ground, samples were taken from elevations known to be attractive to ovipositing females in previous years. As the rising water level covered the usual oviposition sites, samples were taken in a row which extended perpendicularly from the waterline to a vertical elevation as high as 35 cm above the water. Water level was measured from the lowest point in the area using a piezometer or gauge stick. After collection, samples were transported to the laboratory and refrigerated. Eggs were extracted from the samples within 24 hr and species of the eggs determined from their shape and chorionic patterns (Horsfall and Craig 1956). Eggs of *Ae. vexans* were grouped according to age.

RESULTS AND DISCUSSION

Results in both the laboratory and the field indicated that soil from oviposition sites was more attractive to gravid females of *Ae. vexans* than soil from other sites.

Caged mosquitoes exposed to samples of moistened soil in the laboratory deposited a mean of 595 eggs per sample on soil taken from an oviposition site compared to a mean of 87 eggs per sample on soil from an elevated hillside (Table 1). Similarly, when artificially moistened samples of soil were exposed to a natural population of *Ae. vexans*, a mean of 188 eggs per sample was deposited on soil from a known oviposition site compared to a mean of only 10 eggs per sample on soil from a hillside which had never been flooded (Table 2).

Table 1. Distribution of eggs deposited by caged *Aedes vexans* in the laboratory given simultaneous access to samples of soil from a wooded depression that was a known oviposition site and from a wooded hillside that had never been flooded. Urbana, Champaign County, Illinois, 1977.

Source of samples	No. of samples	No. of eggs per sample		
		Maximum	Minimum	Means*±S.D.
Wooded depression	3	628	552	595±39
Wooded hillside	3	144	0	87±76

* The means were significantly different at the 95% level.

The effect of favorable conditioning of soil on oviposition by mosquitoes was also observed under natural conditions (Figure 1). A large population of *Ae. vexans* in a wooded, flood-plain site was ovipositing actively (May 4 and 5) when heavy rains inundated the area, covering the soil at elevations where oviposition had been taking place. Oviposition nearly ceased even though soil above the waterline was sufficiently moist for deposition of eggs. Once the waters receded enough to expose the lower elevations (May 16), oviposition commenced again. Evidently, the soil at lower elevations had been conditioned favorably while more elevated soil was unsuitable.

The natural events which lead to favorable conditioning of the soil and the specific changes caused by these events remain unknown. Certainly the influences of flooding and oviposition should be investigated in future work. The ample evidence in the literature of the influence of chemicals on the selection of oviposition sites by flood-water mosquitoes suggests that conditioning might reflect chemical changes in the soil. Data from the field indicate that ovipositing *Ae. (Ochlerotatus) dorsalis* (Meigen), *Ae. (Och.) sollicitans* (Walker), and *Ae. (Och.) taeniorhynchus* (Wiedemann) are attracted to, or at least tolerate, soil with a high content of inorganic ions (Horsfall 1963, Knight 1965,

Table 2. Deposition of eggs by a natural population of *Aedes vexans* on artificially moistened samples of soil exposed during single nights at a known oviposition site. Samples were from a previously flooded hillside and from an upland depression with a history of flooding. Decatur, Macon County, Illinois, 1977.

Date of exposure	Source of samples	No. of samples	No. of eggs per sample		
			Maximum	Minimum	Mean±S.D.
July 24	Upland depression	2	112	91	102±15
	Hillside	2	17	14	16±2
July 30	Upland depression	2	367	183	275±130
	Hillside	2	11	0	6±6
Total for both nights	Upland depression	4	367	91	188*±126
	Hillside	4	17	0	10*±7

* These means were significantly different at the 95% level.

Petersen and Rees 1966). Ovipositing *Ae. vexans* and *Psorophora (Grabhamia) columbiae* (Dyar and Knab), on the other hand, apparently avoid soil with a high content of inorganic ions (Knight 1965, Petersen and Rees 1966). The possible influence of organic compounds was demonstrated by Ikeshoji and Mulla (1970). Ether extracts of the water overlying oviposition sites of *Ae. (Och.) nigromaculis* (Ludlow) and *Ae. taeniorhynchus* were attractive to ovipositing females of these species.

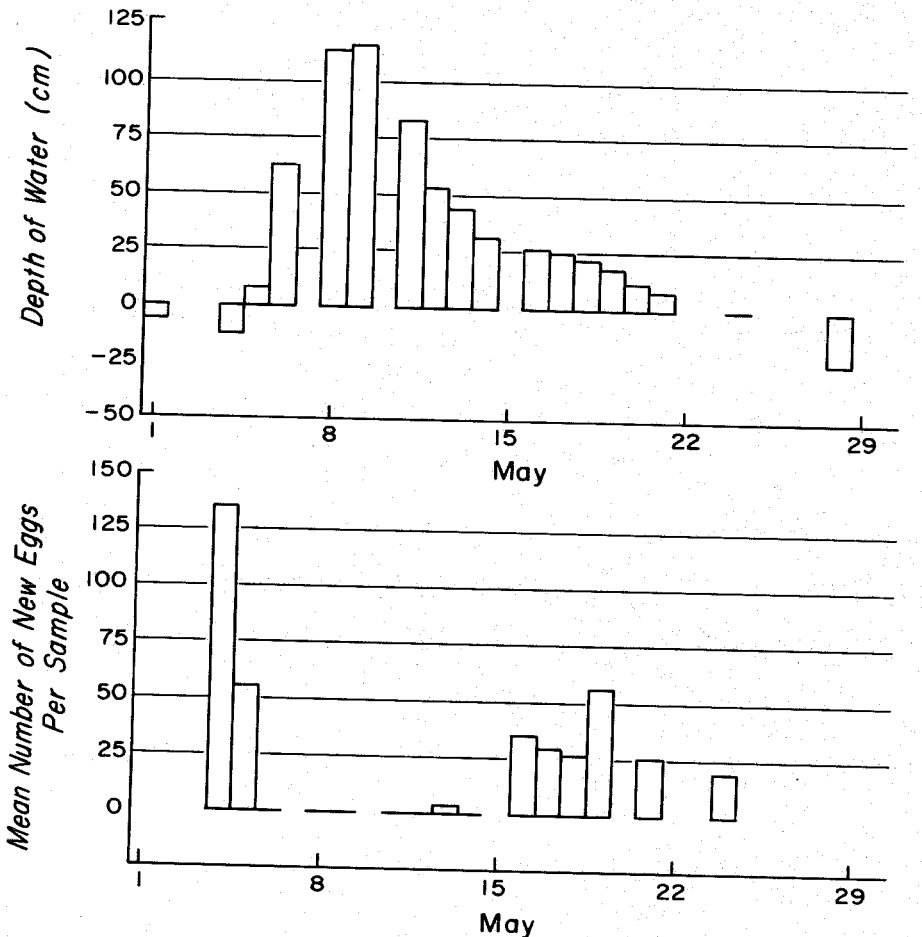

Figure 1. The depth of water in a flood-plain depression before, during, and after a flood and the deposition of eggs by *Aedes vexans*. Freshly deposited (new) eggs were extracted from samples of soil removed from 0 to 35 cm above the waterline. Piatt County, Illinois, 1977.

Whatever the events which favorably condition soil for oviposition or the specific changes in the soil caused by these events, conditioning of the soil has an important role in the life of *Ae. vexans*. Once gravid females arrive in the general vicinity of the oviposition site, a selection of the exact location for deposition of the eggs is made. The process of selection generally results in placing the eggs within a depression where they will subsequently be inundated. The placement of eggs in such depressions is probably mainly dependent on selection by the ovipositing mosquito of soil which is both suitably moist and favorably conditioned. Soil at the bottom of a depression tends to remain moist longer than that of surrounding areas so that in dry periods females tend to gravitate to such areas for oviposition. After rains when large areas are wet, selection of favorably conditioned soil may become important for the successful placement of eggs. The mosquitoes can distinguish between depressions that are likely to flood and other areas on the basis of conditioning of the soil regardless of moistness. If suitable oviposition sites are flooded, then the mosquitoes can hold their eggs until the favorable locations are again exposed.

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