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SUBSTRATE MOISTURE EFFECTS UPON OVIPOSITION IN *AEDES VEXANS*

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ABSTRACT. A laboratory experiment using 12 cohorts of *Aedes vexans* was used to determine the preferred substrate moisture for ovipositing females. Two substrates were used: cheesecloth and sand. On both substrates the extremes (0% and 100% saturation content) were avoided. On cheesecloth, the preferred moisture level was 20% with a decline in preference as the moisture level increased. On sand, there was no clear optimal moisture. Sand moistures between 20 and 80% saturation content were equally preferred. Speculation for a difference in the moisture preference regimes on 2 substrates is included.

INTRODUCTION

Aedes vexans is one of the most widespread pest mosquitoes in the world. Recently in an *AMCA Newsletter*, the regional directors were polled as to which mosquito species constituted the most serious pest in their areas. *Ae. vexans* was given the dubious honor without exception. Within the Midwest, control of this species alone would virtually constitute satisfactory mosquito control for many communities (Siverly 1972).

Since *Aedes* oviposit on the substrate surrounding a body of water, they rely on a rise in water level to hatch their eggs. Several investigators have been able to identify certain necessary requirements for field oviposition sites. Clements (1963) cited substrate moisture as the most fundamental physical attractant. Eggs begin to appear in sites as the water recedes following inundation in the spring and summer. Eggs are laid on soil that is moist but not water-logged, in a zone above the water table (Horsfall et. al 1973). Knight and Baker (1962), using *Ae. taeniorhynchus* and *Ae. sollicitans*, were the first to quantify the amount of substrate moisture in preferred oviposition sites. Peak attractiveness was in a range centered on 85% saturation content. By using similar methods this study quantifies the substrate mois-

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ture preferences of *Ae. vexans* using 2 types of media.

MATERIALS AND METHODS

Ae. vexans used in this study originated from 300 adult females collected in Cook Co., Illinois. The colony was maintained through forced copulation (McDaniel and Horsfall 1957). Standard larval rearing techniques were employed (Craig and VandeHey 1962).

To determine substrate moisture preference, 2 substrates were employed: cheesecloth and sand. Curity® Brand, white, grade 5 cheesecloth was folded to a thickness of 16 layers and provided a basis of comparison with Knight and Baker's (1962) work with *Ae. taeniorhynchus* and *Ae. sollicitans*. The sand used as a substrate was washed, autoclaved, and separated into homogeneous particle sizes by collection in a #18 standard mesh sieve.

A method described by Knight and Baker (1962) was used to determine the percent saturation moisture content. The weight of water needed to saturate one gram of substrate was determined for sand and cheesecloth. Percentages of this saturation value were then used to quantify the amount of water added to each substrate to achieve a specified moisture.

Six substrate moisture levels were offered to 20 gravid *Ae. vexans*. Experiments were always begun 3 days after forced copulation of the females. A cage was constructed of a plastic ring (15 cm in diameter, 4 cm high) with a clear plastic top and a bottom of gray nylon screen (Fig. 1.). A hole in the side which was corked during the experiment allowed the introduction of 20 female mosquitoes. The substrate (either cheesecloth or sand) was held in 57 mm millipore petri dishes. Each substrate was weighed dry, and the amount of distilled water necessary to bring it to a specific percent saturation was added. The six substrate moisture levels 0, 20, 40, 60, 80, and 100% saturation were used. Petri dishes, holding the oviposition substrates, were arranged in a circle. In the center of the circle of six dishes, another dish was placed containing a small piece of cellucotton saturated with honey. This served as a source of carbohydrates during the experiment. The cage of gravid females was placed upon the circle of 6 substrates moistures. The above apparatus was then enclosed within a plastic vegetable crisper with a water saturated paper towel on the bottom. When the lid was placed on the crisper, the entire apparatus soon attained a saturated environment. Tests made by weighing the substrates before and after a 24 hr period within the insectary indicated that none of the substrate moistures changed by more than 5%; therefore, the substrate moisture treatment levels never overlapped one another. The apparatus was then placed in an insectary set at 28° ± 2°C.

After 24 hr, the apparatus was removed from the insectary. The cage was taken off the oviposition substrates. The eggs were removed from each substrate, counted, and recorded. New substrates were prepared and the cage of mosquitoes returned to the insectary within 2 hr. This process was repeated each day during the 5-day oviposition period of *Ae. vexans*.

To remove the effect of position of moisture within the cage, 6 arrangements of the various moisture levels were considered. Each moisture level was placed in each position within the cage once. There were, therefore, 6 possible arrangements or patterns of moisture. Each pattern was used twice making a total of 12 experiments on moisture preference using a single type of substrate.

RESULTS

A total of 345, 4494, 3224, 2244, 1710 and 1499 eggs were laid on the 0, 20, 40, 60, 80 and 100% moisture levels respectively. Assuming the number of eggs oviposited on a moisture level is an indication of the degree of preference which *Ae. vexans* has for that particular moisture, the moisture preference on a cheesecloth substrate is shown in Fig. 2. Zero percent substrate moisture was clearly the least preferred substrate moisture. Preference for

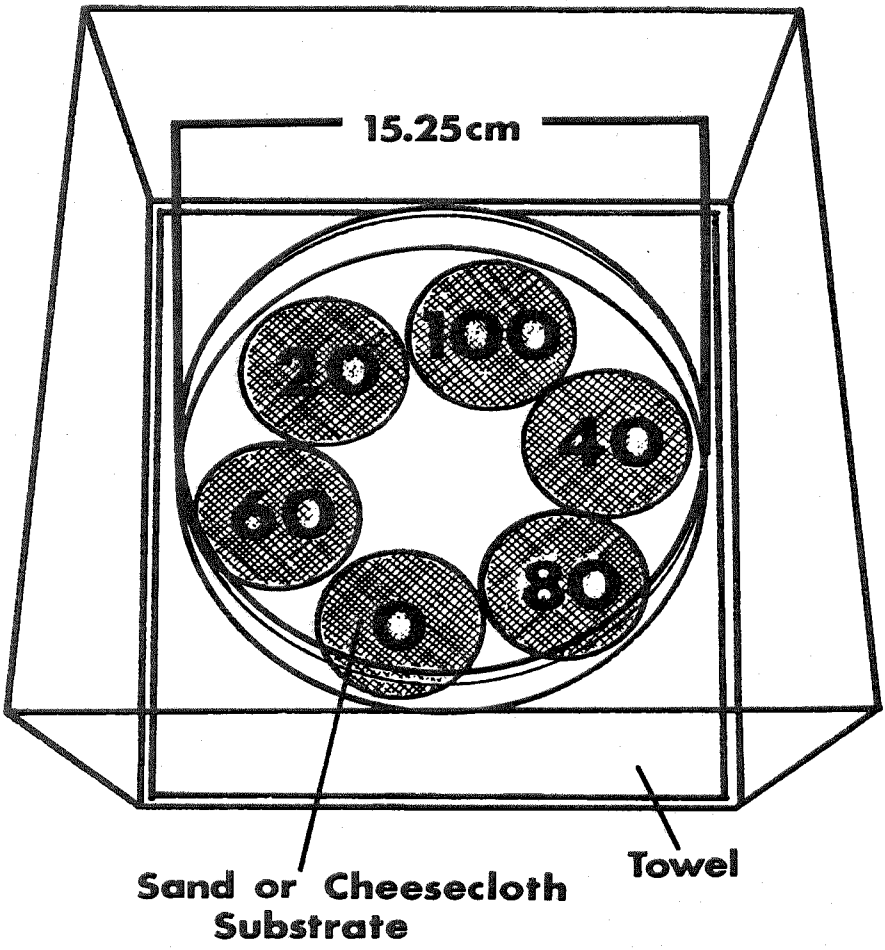

Fig. 1. The experimental apparatus for the substrate moisture experiment. Cheesecloth or sand substrates were placed in 57 mm millipore petri dishes. The cage containing the mosquitoes was placed on top of the oviposition substrates. The entire apparatus was enclosed in a clear plastic vegetable crisper.

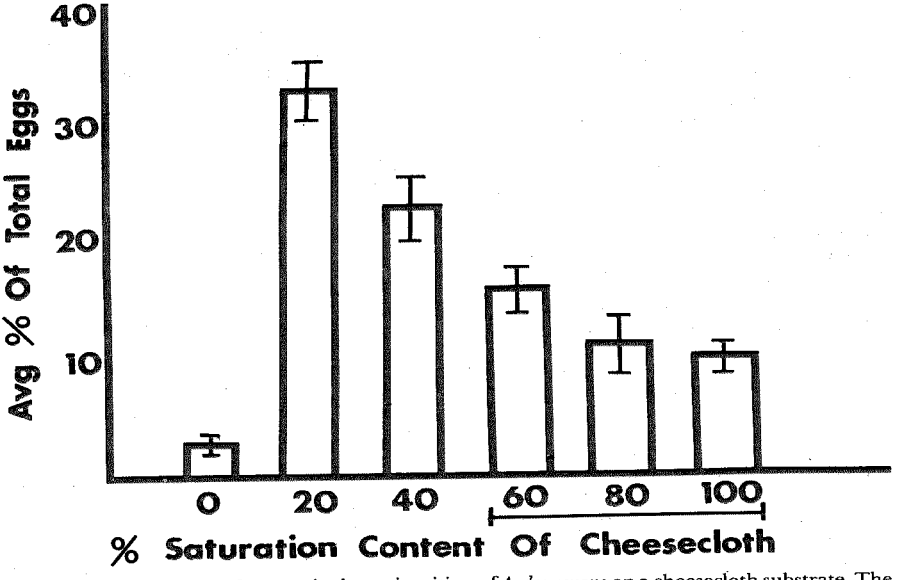

Fig. 2. The moisture preference during oviposition of *Aedes vexans* on a cheesecloth substrate. The interval symbol on each bar is the standard error.

20% moisture is significantly greater than the preference for any other substrate moisture. There is then a gentle decline in preference as substrate moisture approaches saturation.

Statistical analysis involved a Latin square, repeated measure analysis of variance. The analysis had 2 main factors and their interaction: the substrate moisture, the position of moisture within cage, and the moisture by position interaction. Results, as compiled in Table 1, indicated that substrate moisture was highly significant ($P<.001$). Position within the cage and the moisture by position interaction were not significant.

Further analysis using the SNK test indicated that at the 5% significance level, there exists only 1 group of means showing no significant difference. This group included the 100, 80, and 60% moisture levels. Homogeneity is shown by the brackets enclosing the homogeneous moisture levels in Fig. 2. There were significant differences between all other moisture level combinations.

Results for moisture preference when sand was used as the oviposition substrates were quite different from the results using a cheesecloth substrate. A total of 88, 5313, 4449, 4777, 4018 and 952 eggs were laid on the 0, 20, 40, 60, 80 and 100% moisture levels respectively. Experimental

Table 1. The Latin square, repeated measure analysis of variance F table to evaluate the effect of cheesecloth moisture level on oviposition preference in *Aedes vexans*.

Sources	SS	df	MS		F
Moisture	1.343	5	.2684	***	25.47
Position	.0281	5	.0056	ns	0.533
Moisture X Position	.147	20	.00735	ns	0.698
Error	.3161	30	.010536		

analysis was again the Latin square, repeated measure analysis of variance. Table 2 shows that moisture was highly significant ($P<.001$); position was significant at $P<.05$, and the moisture x position interaction was not significant.

Figure 3 shows the percent of total eggs deposited on each substrate moisture when summed over replicate and position. The 0% moisture level again received the lowest percent of total eggs. However, the level of substrate moisture preferred is not as obvious on the sand as upon cheesecloth. The preference for substrate moisture levels 20 through 80% was not significantly different as indicated by the SNK test. Saturation (100%) was the second least preferred substrate moisture.

Table 2. The Latin square, repeated measure analysis of variance F table concerning the effect of sand moisture level on the oviposition preference in *Aedes vexans*.

Sources	SS	df	MS		F
Moisture	2.0147	5	.40297	***	46.80
Position	.1258	5	.0252	*	2.922
Moisture X Position	.1969	20	.00984	ns	1.143
Error	.2584	30	.00861		

DISCUSSION

Results obtained on the effects of substrate moisture on the oviposition preference of *Ae. vexans* support the earlier interpretations of other workers, namely, that moisture is probably the single most important factor acting as an oviposition attractant (Horsfall 1963, Horsfall et al. 1973, Horsfall et al. 1975). The findings reported here indicated that *Ae. vexans* prefers not to oviposit either on dry or saturated substrates.

Preference for 20% moisture content was clearly defined when cheesecloth was the oviposition substrate. On sand, the preferred moisture range extended from 20% to 70% moisture content. The difference in preference regimes resulting

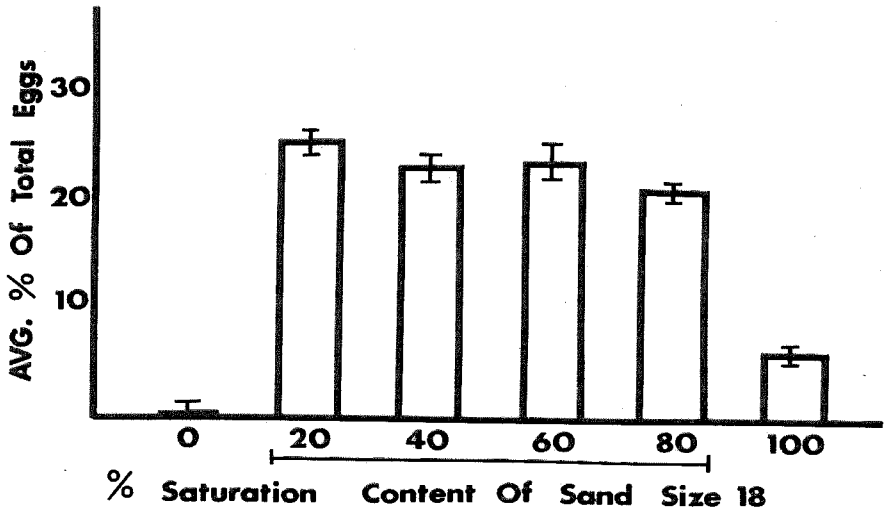

Fig. 3. The moisture preference during oviposition of *Aedes vexans* on a sand substrate. The interval symbol on each bar is the standard error.

from the same moisture levels on cheesecloth and sand suggests that another factor, e.g. texture, may be interacting with the moisture perception of *Ae. vexans*.

From an evolutionary point of view, the placement of eggs upon an optimal substrate moisture should be translatable into a probability of survival. It is known that *Ae. vexans* eggs need some moisture for embryonation (Horsfall et al. 1973), but the optimal range has not been determined. Investigations on the effects of moisture upon embryonation are now in progress. Substrate moisture could also act as a stimulus for an area which has a high probability of experiencing inundation at a later time. However, the correlation between soil moisture and probability of inundation has not been determined.

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