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IE ROLE OF THE SUBSTRATE MOISTURE CONTENT IN THE SELECTION OF OVIPOSITION SITES BY *AEDES TAENIORHYNCHUS* (WIED.) AND *A. SOLLICITANS* (WALK.)[1]

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NTRODUCTION. In common with other dwater mosquitoes, *Aedes* (*Ochlerots*) *sollicitans* (Walk.) and *A.* (*O.*) *iorhynchus* (Wied.) lay their eggs on st soil between the level of maximum ndation and the prevailing water line. ond this, no precise information is ilable about the relationship between substrate moisture content and the seion of an oviposition site by these two quito species.

nowledge of this subject is essential full understanding of the problem of position-site selection and to any effort le towards controlling these mosquito ies through destruction of the egg e. The present study was carried out the purpose of determining under laboory conditions the substrate moisture ent occurring in sites utilized by *sollins* and *taeniorhynchus* for oviposition.

IATERIALS AND METHODS. Under cage litions, both *sollicitans* and *taeniorhyns* readily lay eggs on moist gauze. By ing available to ovipositing females ndomized series of gauze pads, differfrom one another only in the amount of water contained, and by subsequently counting the number of eggs laid on each pad, this capability was used to investigate the relationship between substrate moisture content and the choice of oviposition sites by these two mosquito species.

The strain of *taeniorhynchus* used in this study was obtained from the colony maintained at the Communicable Disease Center's Technical Laboratories, Savannah, Georgia. Since a colonized strain of *sollicitans* is not available, wild females of this species were collected, placed in laboratory cages, given a blood meal, and provided with moist gauze wicks for oviposition sites. After several thousand eggs accumulated, they were washed out of the gauze onto a filter paper and stored in petri dishes over water in a closed desiccator jar. Whenever a cage of *sollicitans* was required for testing, eggs from this supply were hatched and the larvae reared to adults.

To reduce the rate of evaporation of water from the gauze pads during exposure periods, mosquitoes were maintained in cages (20x20x20 inches) of the type described by Chao (1959). The sides of this cage consist of clear sheet acetate attached to the wooden frame with rubber cement. The cage is made addi-

[1] The opinions and assertions contained herein those of the authors and are not to be cond as official or reflecting the views of the y Department or the naval service at large.

tionally tight by tucking the sleeve inside the cage and placing a wooden-framed section of the acetate over the sleeve opening. Between tests, a high relative humidity was maintained in the cage by drawing a soaked gauze wick upward from a beaker of water and tacking the wick to the cage ceiling. At an insectary temperature of 84° F., three of these wicks will maintain the relative humidity in the above-described type of cage between 85 and 90 percent.

The experimental oviposition sites consisted of gauze pads cut to fit snugly into the bottom section of petri dishes (95 mm x 13 mm). Test dishes differed from one another on the basis of the percentage of the weight of the gauze pad due to the contained moisture (percent

by wet weight: $\frac{\text{wt. of water}}{\text{wt. of pad plus water}}$).

To prepare these dishes, the gauze pads were weighed and the weight of water required to give the desired percentage of moisture to each pad was calculated. This amount of distilled water was distributed as evenly as possible over the bottom of the petri dish and the pad was placed in the dish. Just before exposing the dish in the cage, the pad was turned over.

In each experimental trial, a set of eight test dishes (in Experiment 4, a set of 12 test dishes were used) was placed in the mosquito cage for an oviposition period of five hours. In doing this, all sources of moisture were removed from the cage and the eight dishes were placed in randomized order in a circle on the cage floor. Next, a light-proof cover was placed over the cage. The dishes were weighed before and after the exposure period in order to permit determination of the amount of moisture lost by evaporation. To allow the five-hour exposure period to occur during the working day, the experimental cage was kept illuminated at all times except when a trial was in progress. All trials were run at an insectary temperature of 84±2° F.

After the final weighing, each gauze pad was washed separately in a wh basin under a faucet until all eggs w removed. The egg-bearing water was th decanted through a 100-mesh sieve a the eggs were rinsed from the sieve int dish along with a small amount of wa This water with the contained eggs v poured into a Büchner funnel, onto a ter paper printed with a black-lined g (one-fourth inch squares). After the ter was drawn off, the filter paper was moved from the funnel and placed in labeled petri dish. The eggs were coun under a stereomicroscope. In the cou of this study, approximately 850,000 e were counted.

Since different numbers of females w laying each day, it was not possible compare the number of eggs laid in in vidual dishes of a trial set, directly w the number laid in equivalent dishes other sets. Accordingly, the number eggs laid in each dish within a set v converted into a percentage of the to laid in that set. The percentage figu of equivalent dishes for all of the trials each test series were averaged and the percent confidence limits of these avera were determined. In each case, test se consisted of eight trials, except in Exp ment 4 where the test series contained trials.

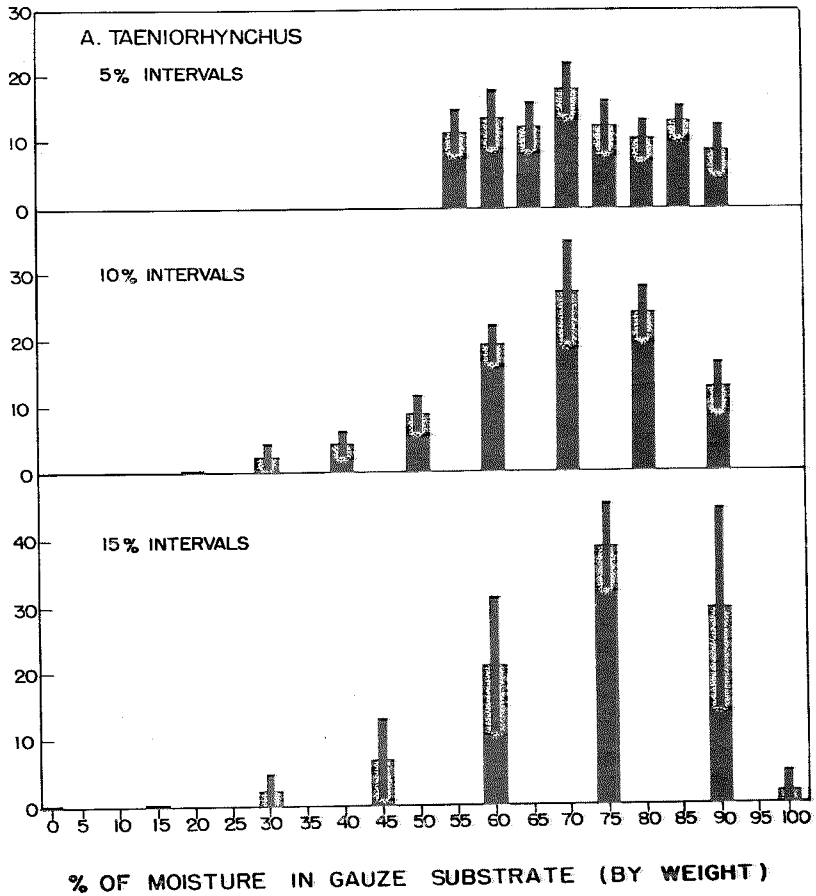
Results. *Experiment 1.* The initial tempt was an effort to determine what p tion of the total substrate moisture ra was selected by the ovipositing fem Accordingly, in the first two trial se (one series for each of the two species) set of eight test dishes had gauze moist contents spaced at 15 percent interv over the range from 0 to 100 perce The results, which are graphically p sented in the lower space of figures 1 a 2, demonstrate a marked preference fo range of substrate moisture contents tween 55 and 95 percent (89 percent all eggs laid were placed in the 60, 75, a 90 percent dishes). There was no mea ingful difference in response between two species.

It is of interest to note that, in t series, a few eggs were deposited on b

dry dish and on the dish containing
er without a gauze pad (100 percent).
s probable that most of these eggs
e laid by immobile dying females and
not represent a "conscious" selection of
position sites.

xperiment 2. Next, to find more pre-
y the limits of the moisture zone ac-
able to the ovipositing females, a test series ranging from 20 to 90 percent substrate moisture with the moisture contents of the gauze pads spaced at 10 percent intervals was conducted, with *taeniorhynchus* only. Here also (Figure 1) the larger percentage, 84 percent, was laid on pads with more than 55 percent of their total weights consisting of water.

Experiment 3. To find out if the accept-



G. 1.—Mean substrate moisture contents selected for oviposition by *Aedes taeniorhynchus.* Smaller
indicates 95 percent confidence limits of the mean. Each test, consisting of eight test sites, was
ated eight times.

able moisture zone could be even more precisely determined, an additional series of trials was conducted with each species. This series extended from 55 to 90 percent, with moisture contents spaced at 5 percent intervals. Although a peak egg deposition appeared to occur at 70 percent with *taeniorhynchus*, the difference in the percent of eggs laid on each of the pads was not striking (Figures 1 and 2). Again, there was no noticeable difference between the two species.

Experiment 4. Because the 5 percent interval series of Experiment 3 was rather uniformly accepted by the ovipositing females, a test series was run consisting of 12 dishes spaced at 5 percent intervals over a range of 40 to 95 percent moisture contents (repeated 10 times; with *taeniorh chus* only). In the previously reported periments, evaporation of moisture fr the dishes occurred unchecked throu out the five-hour exposure period. S sequent checking of moisture loss from gauze pads by the subtraction of final weights from initial weights disclo that moisture contents from 70 percent down were lowered sufficiently during five-hour test period to overlap with initial moisture content of the next lo dish in the test series. In this experim in order to avoid an overlap of ga moisture contents, the pads were brou back to weight at the end of each h of exposure. Although this method not allow the uninterrupted explorat

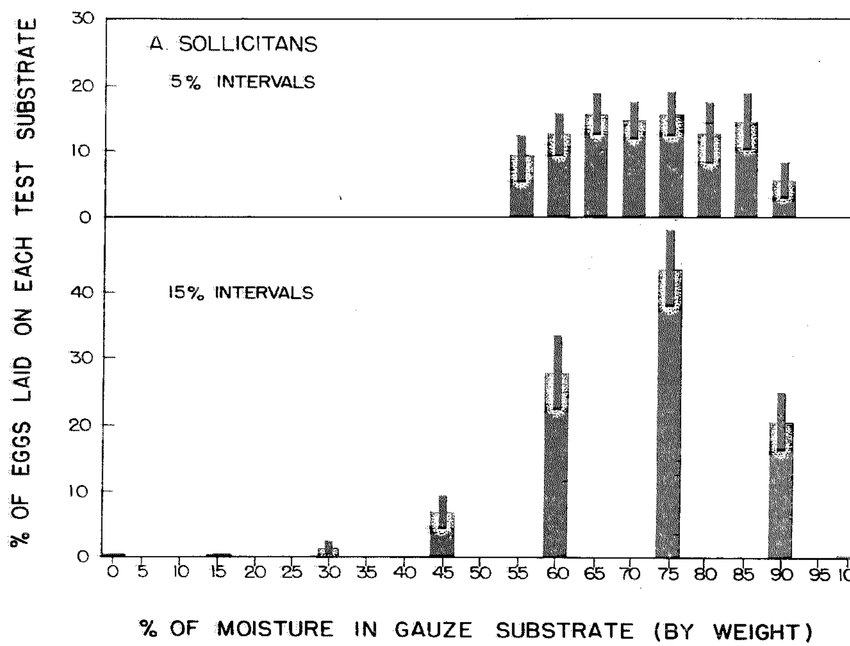
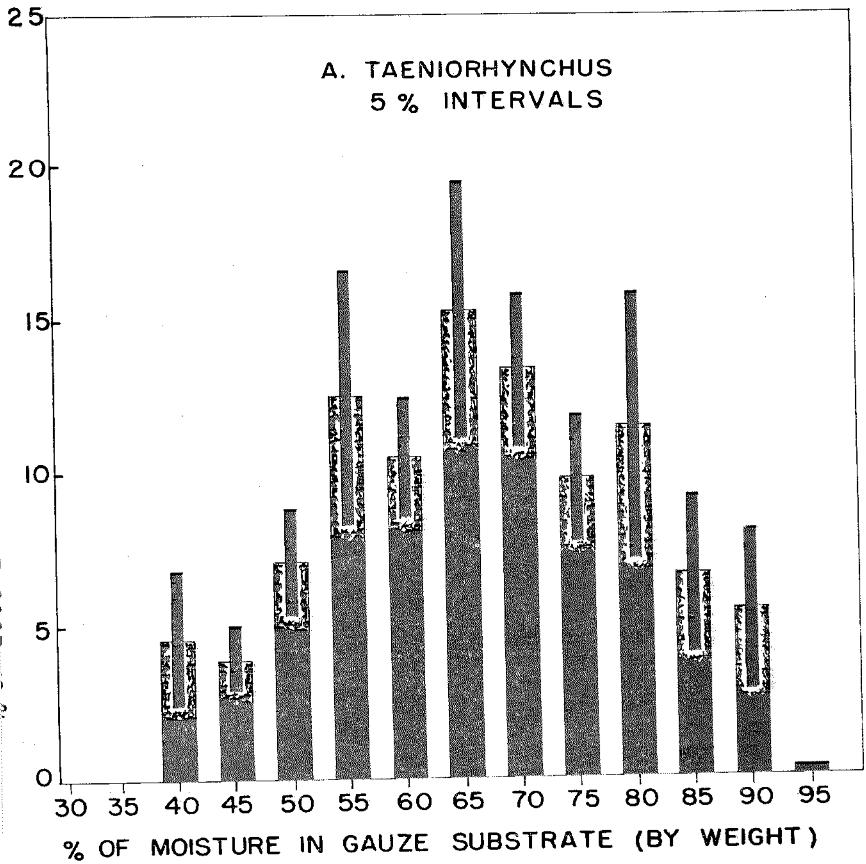


FIG. 2.—Mean substrate moisture contents selected for oviposition by *Aedes sollicitans.* Smaller indicates 95 percent confidence limits of the mean. Each test, consisting of eight test sites, was repe eight times.



IG. 3.—Mean substrate moisture contents selected for oviposition by *Aedes taeniorhynchus* when 12 sites (spaced at 5 percent intervals) were provided. Smaller bar indicates 95 percent confidence its of the mean. Repeated 10 times.

the dishes by ovipositing females, it did least provide a set of test results with- t terminal overlapping substrate mois- e contents. The results of this experi- nt are graphically presented in Figure Although the distribution is incom- te at the lower end, it appears that the rmal curve for substrate moisture con- ts extends from approximately 35 to percent, with an area of rather uniform acceptability occurring from 55 to 80 percent (this zone includes 72 percent of all the eggs deposited in this test series).

Experiment 5. In order to make the data reported here fully meaningful, it is necessary to relate in some manner the gauze-pad moisture contents with actual field-soil moisture contents. This cannot be done from the percentage figures used previously in this paper, since dishes with moisture levels above about 75 percent had free water around the margin of the pads and accordingly were beyond the point of saturation.

Field-soil moisture can be determined in a variety of ways, but apparently the most suitable method is to determine the force with which water is held in the soil and therefore of the force necessary to extract it (Heatwole and Lim, 1961). This provides an expression which is usually called the "soil moisture tension." However, the specialized equipment needed to determine this value was not available.

An expression for soil moisture content used in the analysis for soluble salts in soils is the "saturation moisture content." This is defined "as the maximum amount of water held in the puddled soil without free water collecting in a depression made in the soil mass" (Jackson 1958, 240). The percentage moisture at saturation may then be determined by oven-drying a sample of the puddled soil.

Since the "saturation moisture content" was being routinely determined for soils collected in a concurrent field study of salt marsh mosquito oviposition sites, it was decided to determine the same value for the gauze pads used in the study reported here. This was done by placing a weighed dry gauze pad in a weighed petri dish bottom and dripping distilled water onto the pad until the first drop of free water appeared at the lower edge of the gauze when the dish bottom was steeply slanted. The dish bottom and contained saturated pad were then weighed and the percentage moisture (by wet weight) at saturation of the gauze pad was determined. In five trials, the average value obtained was 77.3 percent (range 76.1–78.2). Using this value as equivalent to a "saturation moisture content" of 100 percent, the percent of "saturation moisture content" has been calculated for each of the gauze-pad moisture contents used in the experiments reported here. This set of conversions is presented in Table 1.

The percent of the "saturation moisture content" can be determined for field sub-

TABLE 1.—Theoretical correlation of the perce[ntage] moisture contents (by wet weight) of the gauze pads used in the experimental work with the "saturation moisture content" (calculated)

Percent moisture content	Percentage of the "saturati[on] moisture conte[nt]"
15.0	19.4
30.0	38.8
40.0	51.7
45.0	58.2
50.0	64.7
55.0	71.1
60.0	77.6
65.0	84.1
70.0	90.5
75.0	97.0
77.3	100.0
80.0	103.5

strates by weighing a sample at the ti[me] of collection, oven-drying it, and then [de]termining the actual "saturation moistu[re] content" as described by Jackson (19[58,] 240).

By consulting Table 1, it can be seen th[at] the area of maximum preference of t[he] ovipositing females, about 55 to 80 perce[nt] substrate moisture (by wet weight), li[es] above 70 percent of the "saturation mo[is]ture content."

Experiment 6. In the previous expe[ri]ments, we sometimes found abnorm[al] eggs on gauze pads which had had lo[w] initial moisture contents. In some cas[es] these eggs had failed to darken normall[y;] in others, even though black they we[re] crumpled, flattened, or even ruptured. [It] seemed quite probable that such abno[r]malities were the results of insufficie[nt] moisture in the substrata bearing the eg[gs].

To determine the fate of eggs laid [on] substrata with low initial moisture leve[ls,] an experiment was conducted in which [a] set of six test dishes, each with moistu[re] contents of 25 percent, was placed in [a] cage of *taeniorhynchus* for 30 minutes [at] the beginning of the third hour of a fiv[e-]hour oviposition period. These dishes we[re] weighed before and after the 30-minu[te] exposure period, at which time five of t[he] dishes were covered. The sixth dish w[as] left uncovered and allowed to dry at roo[m] mperature. Each 30 minutes thereafter, l dishes were weighed and each time, ne more dish was left uncovered. Mois-re was lost very slowly when the dishes ere covered but quickly dropped to below percent when left open. By opening additional dish each 30 minutes, it was ossible to observe the effects of aging on the survival of eggs on substrata with tremely low moisture contents. The ex-riment was terminated at the expiration 150 minutes from the time when the shes were removed from the oviposition ge. At this time, the gauze pad of each sh was washed and the contained eggs ere recovered and examined for condi-on.

Under the conditions of this experiment, e minimal gauze-pad moisture level cessary for normal egg development as in the range of 13–16 percent (17–21 rcent of the "saturation moisture con-nt"). If the moisture content dropped low this level before the darkening ocess was completed (approximately 75 inutes from the time of laying), no rther darkening occurred and the eggs came flattened. Upon re-exposure to oisture at the time the eggs were washed om the gauze pads, these white or ay eggs quickly regained their shape. owever, completion of darkening did ot occur. If the gauze-pad moisture con-nt did not drop below the minimal vel until after the eggs had blackened, llapse occurred as previously, but the gs did not recover their normal shape hen exposed to moisture on being washed om the gauze. It would appear from is that moisture is absorbed less readily eggs which have completed darkening.

DISCUSSION. From the laboratory study ported here, it seems probable that *eniorhynchus* and *sollicitans* normally se-ct soils for oviposition with moisture ntents above 70 percent of the "satura-on moisture content." Such high soil oisture levels, particularly when com-ned with a cover of living and dead or-nic material, undoubtedly produce high lative humidities immediately above the il. Experience in the laboratory has indicated that both of these species have high moisture requirements, surviving best at relative humidities of 85 to 95 percent. In view of this, it is thought likely that the selection of highly moist soils is a reaction by the ovipositing females to the moist atmosphere which normally will occur over such soil, rather than because these places favor the survival of the eggs and larvae.

It could be said that the selection of moist soils for oviposition is not done solely for the comfort of the gravid females because these females do not remain at all times just above moist soil. However, the changing physiological state of the adult is probably the controlling factor here. Bar-Zeev (1960) showed for *Aedes aegypti* (L.) that properly conditioned gravid females gave no reaction to the air close to a wet surface and even moderately avoided a wet surface. At first glance, his conclusions appear to contradict the observations made here. However, he further observed that in spite of deferred opportunity to ovipost, gravid females did not begin to lay eggs immediately when the opportunity was offered. He concluded from this that gravid females probably must get into a physiological "egg-laying state," and that it is only when such a state has been reached that the mosquitoes are attracted to a wet surface to oviposit. Our own observations confirm this in that on several occasions when test dish sets were examined at the end of the first and second hours of the five-hour test period, very few eggs were found.

It can be readily understood that the selection of highly moist soils for oviposition sites has an important survival value to these mosquitoes. As shown by Telford (1957), Beckel (1958), and Rosay (1959), newly laid eggs are freely permeable to water. After the eggs blacken, they lose water more readily than they regain it, and finally a stage in embryonic development is reached when the eggs are essentially waterproof. If the substrate becomes too dry between oviposition and this time, water is lost and and the eggs collapse. If desiccation continues beyond the time of loss of permeability, the process of water loss is irreversible and the eggs are destroyed.

A damp oviposition site has the further survival value of being an area that is likely to be reflooded before adjacent drier areas, a situation which insures the subsequent hatching and development of the larvae.

SUMMARY. The capability of *Aedes sollicitans* and *A. taeniorhynchus* to lay eggs on moist gauze was used to investigate in the laboratory the relationship between substrate moisture content and the choice of oviposition sites by these two mosquito species. Results obtained indicate that gauze pads with moisture contents of less than 45 percent (by wet weight) are unattractive to ovipositing females of *sollicitans* and *taeniorhynchus* (at least in the presence of gauze pads with higher moisture contents). Conversely, pads with moisture contents above 45 percent are definitely attractive, with the peak attractiveness apparently occurring in the region of 65 percent. Gauze-pad moisture contents were related to actual field-soil moisture contents by determining a moisture content equivalent to the soil "saturation moisture content." Correlating this determination with the results obtained from the experimental work, it seems probable that these two species normally select soils for oviposition with moisture contents above 70 percent of the "saturation moisture content."

Substrata with moisture contents as lo as 17 percent of the "saturation moistu content" will support normal egg dev opment. However, such dry substrata a highly susceptible to desiccation, a much wetter substrata are necessary if fu egg development and survival are to ensured.

Eggs were not laid, "by design" at lea upon free water surfaces. The two speci did not differ significantly in their reacti to substrate moisture. Probable causes f the results obtained are discussed.

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The secretary reminds all subscribers to *Mosquito News* and all AMCA membe that at the last annual meeting it was voted to increase the dues and cost of subscripti to $8.00, beginning January 1, 1963. This and other important action taken at t Galveston meeting are fully described in the *Minutes*, pages 188–213 in the June, 196 Number (vol. 22, #2).