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ON THE BIONOMICS OF BROMELIAD-INHABITING MOSQUITOES. I. SOME FACTORS INFLUENCING OVIPOSITION BY *WYEOMYIA VANDUZEEI*[1]

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ABSTRACT. In southern Florida the immature stages of the mosquito *Wyeomyia vanduzeei* Dyar and Knab are found predominantly in water held in the leaf axils of bromeliads. As part of a study of the factors controlling mosquito population size, we developed a method for sampling the immature stages (eggs, larvae and pupae) and used it here in laboratory studies to determine the influence of plant size and presence of water in the leaf axils on the number of eggs laid. The presence of organic infusion and of organic debris in bromeliad leaf axils did not appear to be of any importance in influencing oviposition.

INTRODUCTION. In an attempt to determine the factors which regulate population size in a natural population of *Wyeomyia vanduzeei*, we made routine weekly population counts since summer 1973 of the immature stages of the mosquito. These counts have given us estimates of standing crop (in the sense of Odum and Odum 1959) and of the way in which standing crop changes in time. There are 3 distinct methods of investigating the influences of a variety of factors on the size of the standing crop; these are (1) by direct experimentation in the field, (2) by experimentation in the laboratory and (3) by correlation of field observations with measurements of possibly influential factors. We have used a combination of all 3 methods.

Our field study makes use of the fact that the only naturally-occurring tank bromeliad in our study area, *Tillandsia utriculata* L., does not require attachment by its roots to a substrate in order to grow. We have tied living *T. utriculata* plants with nylon cord and suspended them from the limbs of trees and shrubs. Bromeliads suspended in this way may readily be removed and replaced, and they make ideal sampling units. Oviposition by *W. vanduzeei* takes place in them and the eggs are able to develop to the adult stage,

SAMPLING METHOD. The apparatus

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illustrated in Fig. 1 is used as follows: A bromeliad to be sampled is inverted over the aluminum cone and dunked into the water a standard (20) number of times. The bromeliad is then removed from the water drum. The aluminum cone is slowly lifted out of the water by its handles, allowing the water contained in it to drain through the hardware cloth screen. Water from a wash bottle is used to ensure that all the solid material within the aluminum cone is washed onto the hardware cloth screen. The Plexiglas tube with the hardware cloth screen is removed from the cone, inverted over a petri dish, and its contents washed into the petri dish by water from a wash bottle. The immature mosquitoes are picked out of the petri dish using a fine glass dropper, or a larger one for pupae, under a binocular microscope, identified and counted.

CAGE TESTS. The 4 tests described below used a cage of size appromixately 35 cm deep, 63 cm wide and 48 cm high, the top, back and sides of fiberglass mosquito screen, the floor of Formica®, the front of Plexiglas® with, below the center, a sliding door with a cloth sleeve. The cage was fitted with 4 glass vials, each with a cotton wick, holding 5% sugar water as a food source. A blood meal was offered daily to the female mosquitoes in the form of a laboratory mouse held in a hardware cloth restraining cage. Newly-emerged mosquitoes from wild-caught larvae or pupae were placed in the cage daily to maintain a population of adults of mixed age. The cage was placed on a bench in the laboratory; no special attempt was made to control temperature, humidity or light, but day length was approximately normal insofar as no artificial light was used in the hours between sunset and sunrise. The tests were conducted between May and August 1975. We recorded the number of eggs recovered as described below. Sometimes a small proportion of the eggs recovered had hatched, resulting in a few instar I larvae, but no distinction was made in the tabulated results between hatched and unhatched eggs.

PLANT SIZE. *T. utriculata* plants in pairs of discrete size difference were selected, the size measured in two ways: (1) the water-holding capacity of the leaf axils in ml and (2) the length of the longest leaf in cm. The plants were hosed out to remove as much as possible of the organic material of the leaf axils. Both plants were tied with nylon cord, and one was hung in the cage at the left side, the other at the right side, at approximately equal height from the top of the cage and about 40 cm apart, median axis to median axis. Tap water was added to the central whorl of each plant until it began to overflow from the plant, when all leaf axils capable of holding water did so. The plants were left in the cage for 72 hr, the mosquito eggs contained were collected by the apparatus described above and the plants, after being hosed out again, were replaced in the cage but with the opposite arrangement. After a further 72 hr the plants were sampled again for mosquito eggs, but instead of being replaced in the cage, 2 new plants were used. In this way a total of 4 pairs of plants was used, and each plant of each pair was hung for 72 hr at one side of the cage followed by 72 hr at the other. The sizes of the plants of each pair are recorded in Table 1. By using 4 pairs of plants and revers-

Table 1. Number of eggs laid in large plants and in small plants when both were available.

Large plants			Small plants	
Size	No. of eggs		No. of eggs	Size
60 ml, 31 cm	122 107	Pair 1	0 1	9 ml, 20 cm
160 ml, 40 cm	62 89	Pair 2	27 24	22 ml, 27 cm
165 ml, 42 cm	98 117	Pair 3	27 11	50 ml, 29 cm
170 ml, 43 cm	141 131	Pair 4	0 0	9 ml, 22 cm

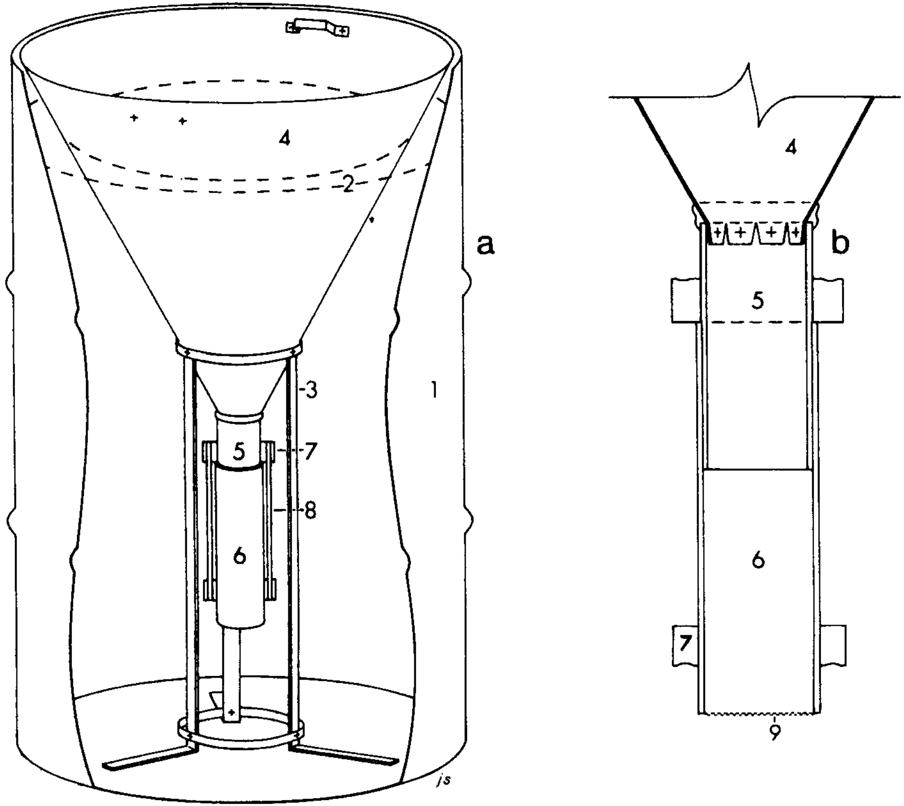

Fig. 1. (a) Apparatus for sampling contents of bromeliads; 1: water drum, 2: water level, 3: stand, 4: aluminum cone with (5) tubular Plexiglas extension, 6: removable Plexiglas tube, 7: Plexiglas lug to hold (8) rubber band; (b) Lower part of aluminum cone with items numbered as in (a) and showing (9) end of removable Plexiglas tube with 100-mesh stainless steel hardware cloth.

ing the positions of the plants within the cage we hoped to eliminate nonrandom variability associated with position of the plants in the cage and any differences between the plants other than size.

Field studies had already shown that larger plants generally contained more eggs. The laboratory test verified that more eggs are laid in larger plants, as indicated in Table 1. We used a t-test for the paired data (Snedecor and Cochran 1967) to demonstrate the significance level

$$t_d = \frac{\Sigma\ (x-y)/n}{SD\ x-y/\sqrt{n}}$$

We calculated $t_d=7.487$ where a 1-tailed $t_{0.001[7]}=4.785$, $P>99.9\%$ that there were significantly more eggs laid in the larger plants than in the smaller plants under the conditions of the test.

PRESENCE OF WATER. In field samples we had often found fewer eggs in plants with little water in the leaf axils. We suspected that more eggs are laid in plants with a lot of water in the leaf axils. A cage test was conducted, in many respects the same as the test for the influence of plant size, except that we used only 2 plants, of the same size, and reversed their positions in the cage once. We hosed out both plants and dried them inverted in a dry cupboard for 24 hrs. Both plants were then put into the cage and one plant was filled to overflowing with tap water. The plants were left in the cage for 72 hrs, the eggs removed, both plants hosed out, then dried for 24 hrs; both plants were replaced in the cage, but this time the plant which had previously been without water was now filled with water and the other left dry. In this way each plant was twice placed at the left side of the cage, once as a "wet" plant and once as a "dry" plant. We hoped by these means to eliminate nonrandom variability associated with position of the plants in the cage and any differences between the plants. Between each replicated test there was a period of 24 hrs when no plants were in the cage. We performed only 4 replicates of the test because the results, shown in Table 2, were clear-cut.

Table 2. Number of eggs laid in "wet" plants and in "dry" plants when both were available.

No. in "wet" plants	No. in "dry" plants
583	0
419	8
284	48
121	1

The calculated t_d value of 3.330 where a 1-tailed $t_{0.025[3]}=3.182$, $P>97.5\%$ that there were significantly more eggs laid in the plants which had leaf axils containing water, under the conditions of the test.

PRESENCE OF ORGANIC INFUSION. Leaf axils of bromeliads in the field contain an organic infusion of the breakdown products of plant exudates, debris and the products of the various animals present in the leaf axils. We had no field data to support a contention that the presence of an organic infusion would lead to any change in the number of eggs laid but thought it worthwhile to find out.

Our cage test used a single pair of plants and organic infusion from bromeliad leaf axils filtered through glass wool as against tap water. Each plant was placed 4 times at the left side of the cage, 2 of the 4 times with organic infusion, the other 2 times with tap water. The plants were hosed out each time before filling with tap water or organic infusion and placement in the cage. By these means we hoped to eliminate nonrandom variability due to position of the plants in the cage and any differences between the plants.

The results are shown in Table 3. The calculated t_d value of -0.895, where a 2-tailed $t_{0.05[7]}=2.365$ indicates no significant difference between the number of eggs laid in plants with organic infusion in the leaf axils as against plants with tap water.

PRESENCE OF DEBRIS. In certain locations and particularly at certain times of year no water is directly visible in the leaf axils of plants in the field because organic debris, seeds, dead leaves and twigs of oak and other trees, occupy much of the

Table 3. Number of eggs laid in plants with organic infusion and in plants with tap water when both are available.

No. in plants with organic infusion	No. in plants with tap water
178	145
73	189
21	19
183	239
39	15
123	84
44	58
64	111

volume of the leaf axils. We had no field data to suggest either a diminution or an increase in the number of mosquito eggs present in the leaf axils of plants with a lot of organic debris, but we wished to discover what effect the debris would have on oviposition.

The cage test performed was identical to that above where we used organic infusion, except that when using organic infusion with debris we had stuffed all leaf axils with dead oak leaves so that no water was visible in the leaf axils even when these were filled to overflowing.

The results are shown in Table 4. The calculated t_d value of 0.982, where a 2-tailed $t_{0.05[7]}=2.365$, indicates no significant difference in the number of eggs laid in the leaf axils of plants with organic infusion plus debris as against plants with tap water.

DISCUSSION. The results obtained determined that *W. vanduzeei* lays fewer eggs in smaller bromeliads when larger bromeliads are available and fewer eggs in bromeliads without water in the leaf axils when bromeliads with water in the leaf axils are available, but that the presence of organic infusion or of organic infusion plus organic debris are not determinants of the number of eggs laid. This is so under the conditions of our cage experiments, and it is not unreasonable to suggest a similar effect in the field, especially when supportive data are available.

The oviposition behavior of several mosquito species is influenced by the presence of organic infusion (Clements 1963). It may be suggested that the apparent lack of response of *W. vanduzeei* to organic infusion was because such a response would be unnecessary. The bromeliad *T. utriculata* grows as an epiphyte and in situations where the presence of organic infusion in the leaf axils is assured.

De Zulueta (1950) noted a response by ovipositing females of *Culex pipiens quinquefasciatus* to infusion and was able to obtain oviposition on distilled water in a vessel by floating a petri dish containing infusion on the surface of the vessel; the infusion attracted females to the surrounding distilled water. In the cage tests with *W. vanduzeei* there was no evidence that the presence of a water-filled bromeliad led females to oviposit in neighboring dry bromeliads.

The presence of much organic debris in bromeliad leaf axils did not appear to be a physical barrier to oviposition by *W. vanduzeei*, nor did it appear to enhance oviposition by providing a greater stimulus than did water alone.

It remains to be determined whether the attraction of wet bromeliads to ovipositing *W. vanduzeei* is visual, chemosensory, or a combination of both.

Table 4. Number of eggs laid in plants with organic infusion plus organic debris and in plants with tap water when both were available.

No. in plants with organic debris	No. in plants with tap water
64	99
71	37
161	48
31	13
42	86
146	38
40	67
41	38

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