

upon insecticide susceptibility test data from one or even several collections of *Cx. quinquefasciatus* to be accurately representative of an area the size of a county, or even smaller. Although insecticide tolerance represents a warning signal to the mosquito control program and assists the field worker in more effectively evaluating control procedures, inadequate sampling and testing may lead either to a false sense of security or unnecessary alarm. All the more dangerous are attempts to extrapolate the known insecticide susceptibility status of the species in one part of a state to another part without testing.

In spite of the presence of chlorpyrifos and malathion tolerance in a number of strains, due to their use as a larvicide in ditches and as an adulticide, respectively, good control of *Cx. quinquefasciatus* in Brazoria and Galveston Counties is still being obtained at recommended dosages.

**ACKNOWLEDGMENTS.** The authors extend special thanks to Mr. W. B. Moon, Director, and Becky Smith of the Galveston County Mosquito Control District, and to Mr. J. C. McNeill, IV, Director of the Brazoria County Mosquito Control District, for kindly providing the initial material used in this study.

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## ON THE BIONOMICS OF BROMELIAD-INHABITING MOSQUITOES. IV. EGG MORTALITY OF *WYEOMYIA VANDUZEEI* CAUSED BY RAINFALL<sup>1</sup>

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**ABSTRACT.** In southern Florida the immature stages of the mosquito *Wyeomyia vanduzeei* Dyar and Knab are found in water held in the leaf axils of bromeliads. Correlation of data resulting from weekly estimates of the numbers of eggs, larvae and pupae vs.

rainfall data indicated possible loss of eggs, but not of larvae or pupae, in heavy rain. This was confirmed by direct experimentation. Heavy rainfall appears to be an important mortality factor for the eggs of this mosquito.

**INTRODUCTION.** We have demonstrated (Frank et al. 1976) that presence of water in the leaf axils of tank bromeliads is a determinant of oviposition by *Wyeomyia vanduzeei*. Because the presence of water

in leaf axils is necessarily determined by the quantity of recent rainfall, it would be logical to examine the relationship between, on the one hand, rainfall quantity and, on the other, change in number of eggs of *Wy. vanduzeei*. We wished to examine this relationship in the natural population in our study area (Frank et al. 1976),

<sup>1</sup> Supported by NIH Research Grants No. AI-06587 and FR-05553

believing this approach to give a better indication of the course of events in nature than could be simulated by laboratory experiments alone.

**PROCEDURES.** Samples of eggs, and of larvae and pupae, were taken weekly and nominally from 10 bromeliads, for a period of over 2 years. That is, the contents of 10 bromeliads were to be sampled and the selection of these bromeliads was made on a random but cyclical basis so that the contents of each plant (of the total 200 plants of the series) were sampled once every 20 weeks. However, if the bromeliad to be sampled was dead, and therefore contained no water and no immature *Wyeomyia*, the number of plants sampled was necessarily reduced below the nominal 10. The average number of plants sampled each week was greater than 9. Any dead plant due to be sampled was replaced, at the time of sampling, by a plant of standard size, brought into the study area from outside, so that the "new" plant would be ready for sampling after a further 20 weeks.

The apparatus described by Frank et al. (1976) was used for washing out the contents of the plants for sampling. Eggs, the 4 different larval instars, and pupae of *Wy. vanduzeei* and of *Wy. medioalbipes* Lutz were identified and recorded separately for each plant sampled.

Because the contents of the bromeliads were removed at the time of sampling and were not replaced, "new" plants were also washed out before placement in the study area. All plants sampled had thus been washed out 20 weeks previously and subsequently "colonized" by eggs laid by females of the natural *Wyeomyia* population in the study area. This 20-week interval would theoretically make a week-to-week comparison of our sample data more reliable by reducing non-random variance due to "colonization" at different times.

Mortality of plants did not appear to be excessive when compared roughly with mortality of plants growing in other areas. For example, where the same bromeliad species (*Tillandsia utriculata* L.) grows on

oak trees in the vicinity of our laboratory, storms blow down considerable numbers of the plants by breaking dead oak branches to which the bromeliads are attached, and bromeliads on the ground are eaten by rabbits. In our study area bromeliads do not naturally grow at ground level, perhaps partly because the entire area is subject to flooding by saline water, but also because plants which are placed on the ground are eaten by rabbits.

Most mortality of plants in our study population appeared to have been caused by saline exudates from the leaves of the black mangrove (*Avicennia nitida* Jacq.). A few black mangrove trees grow in our study area and in some instances their canopies overhung the bromeliads we had suspended from the branches of the much more numerous white mangrove (*Laguncularia racemosa* (L.)) and buttonwood (*Conocarpus erectus* L.). When we finally suspected the effects of the exudates from black mangrove, we trimmed the branches of these trees so that exudates from their leaves could not be washed into the bromeliads by rainfall; this resulted in much reduced mortality of the bromeliads.

Daily rainfall records have been kept at Florida Medical Entomology Laboratory for the past 17 years. The laboratory rain gauge is about 0.35 mile from our study area. Using several rain gauges of standard type we measured during a period of 6 weeks: (1) rainfall at the laboratory, (2) rainfall in the open in the study area and (3) rainfall under the tree canopy in the study area. While rainfall in the study area undoubtedly differs from that at the laboratory, the differences observed during our series of measurements were not significant, so that we felt justified in using the rainfall records compiled at the laboratory. The rainfall under the tree canopy is significantly less than that in the open, especially in low-intensity rainfall. These differences will be reported elsewhere but are ignored in the following analysis.

Because our samples of the contents of bromeliads are taken weekly, we have examined their relationship with total

weekly rainfall instead of daily rainfall. The size of the *Wyeomyia* population in the study area varies seasonally. It is obviously unrealistic to expect that the number of *Wyeomyia* eggs (or larvae) in a bromeliad is dependent solely on the rainfall of the preceding 7 days, therefore it is not acceptable to calculate a regression of actual number of eggs taken in samples vs. rainfall. The number of eggs laid must depend first upon the size of the mosquito population and secondly upon the condition of the bromeliads. The condition of the bromeliads may be modified by the rainfall of the previous 7 days. Rainfall might affect, directly or indirectly, survival of adult mosquitoes. The number of eggs present in plants depends on the number of eggs laid during the previous 7 days, but also depends upon the number lost during that time which, in turn, must depend upon mortality and hatching of eggs, both of which might be affected by rainfall.

Therefore we compared the proportional increase in number of *Wyeomyia* eggs with rainfall of the 7 days preceding the sample. This comparison was made as a correlation, since correlation techniques are generally more appropriate where estimates of both independent and dependent variables are subject to error. The values for rainfall (X) thus consisted of total rainfall in mm for the 7 days immediately preceding each sample, taken always on a Wednesday. The values for proportional increase in number of eggs (Ye) consisted of the estimate of total number of eggs per 10 plants for week  $n \div$  estimate of total number of eggs per 10 plants for week  $n-1$ . These data are given in table 1 for a period of 81 weeks from 15 August 1973 (week 8) to 26 February 1975 (week 88) inclusive, resulting in a total of 81 values. In the same table are given equivalent values for each of the 4 larval instars and for the pupae.

The correlation coefficients were as follows: eggs:  $-0.238$ ; instar I larvae:  $-0.062$ ; instar II  $-0.014$ ; instar III:  $-0.036$ ; instar IV:  $-0.050$ ; pupae:  $-0.006$ . While it is interesting that all of the coefficients were negative, only the

value ( $-0.238$ ) calculated for the correlation of proportional increase in number of eggs was significant ( $P < 0.05$ ) in a 2-tailed test of significance. It is thus evident that the number of *Wy. vanduzeei* eggs is likely to be lower after a week in which heavy rain fell than before that week. On the other hand numbers of larvae or pupae are not significantly altered at the end of a week in which heavy rain fell.

Several reasons could be advanced to account for the reduced number of eggs sampled after a week with heavy rain: (1) that eggs stranded above the water line in the plants might be caused to hatch by the rain; (2) that rain washes (flushes) eggs out of the plants; (3) that the impact of raindrops splashes eggs out of the plants; (4) that mortality of adult females is increased by heavy rain or that for other reasons oviposition is lower during weeks with heavy rain. All of these reasons suggest that water held in the leaf axils was seldom so little that oviposition in the study area was inhibited by this factor or, if this effect did occur in the time period considered our present analysis does not demonstrate it.

If eggs stranded above the water line were caused to hatch by rainfall, there should be a corresponding increase in number of larvae, but there is no evidence of increase in numbers of any of the larval instars. Possible interpretations thus seem to be reduced largely to suggestions (2), (3) and (4) above, although (1) cannot be ruled out entirely, if, for example, eggs were caused to hatch by rainfall, but instar I larvae were washed out of the plants.

We carried out 2 simple experiments to test some of the possibilities. On 9 June 1975 we had available 20 spare bromeliads of standard size. At 3:45 p.m. it appeared that a thunderstorm was about to occur. The 20 plants were hosed out quickly to remove most of their organic contents, then taken into the laboratory. We had available a number of live eggs and larvae of *Wy. vanduzeei* in rearing dishes in the laboratory. We counted out 10 live instar III larvae into each of 20 vials, then washed the contents of each vial into the

Table 1. Total rainfall in mm in the 7 days preceding the sample (X), and proportional increase over previous week of no. eggs (Ye), instar I larvae (Yi), instar II larvae (Yii), instar III larvae (Yiii), instar IV larvae (Yiv), and pupae (Yp) of *Wyeomyia vanduzeei*.

Week	X	Ye	Yi	Yii	Yiii	Yiv	Yp	Week	X	Ye	Yi	Yii	Yiii	Yiv	Yp
8	0	0.81	1.32	3.42	0.80	1.15	0.6	49	1.0	2.17	1.01	0.77	0.88	0.90	1.62
9	52.0	1.20	2.84	1.39	1.03	1.30	1.33	50	19.2	0.17	0.48	1.02	0.73	0.85	0.76
10	59.3	1.25	1.70	1.08	2.06	2.76	1.97	51	1.6	2.46	2.00	1.94	1.15	1.86	1.00
11	20.6	0.58	0.12	0.90	0.72	0.63	0.72	52	14.8	2.14	1.20	0.42	1.15	1.25	1.70
12	2.7	2.36	18.70	2.01	2.38	1.53	1.62	53	123.5	0.30	2.04	1.80	1.91	1.63	1.11
13	84.0	1.08	1.00	1.31	1.17	0.88	0.61	54	78.3	1.61	0.50	0.27	0.16	0.28	0.05
14	69.8	0.17	0.20	0.45	0.62	0.45	0.50	55	13.8	0.99	0.58	3.77	4.47	1.80	5.00
15	12.3	4.75	2.82	4.07	2.87	3.37	4.00	56	29.2	1.10	0.58	0.84	1.19	1.78	7.00
16	70.9	0.62	0.92	0.56	0.76	0.77	0.62	57	36.6	1.25	0.75	0.65	0.61	0.65	0.37
17	8.2	2.28	1.29	0.91	1.03	1.43	1.00	58	116.2	0.86	0.60	0.56	0.67	1.73	1.84
18	48.8	0.31	2.16	1.65	1.66	1.42	2.20	59	27.4	1.18	4.73	3.35	0.89	0.48	0.75
19	1.7	1.58	0.68	0.64	0.51	0.82	1.00	60	3.2	1.50	1.36	0.74	1.54	1.03	1.00
20	14.0	0.67	0.86	1.15	1.40	1.12	0.18	61	45.8	0.65	0.68	0.97	1.03	1.09	1.05
21	0	2.15	0.63	0.47	0.33	0.80	0.25	62	52.2	1.29	1.27	2.34	2.13	1.82	1.00
22	28.7	0.72	1.72	1.96	1.41	0.84	2.00	63	3.5	1.07	1.62	0.89	0.79	1.08	1.05
23	3.0	0.52	0.73	0.85	1.50	1.36	2.00	64	7.0	0.56	1.00	0.91	1.12	0.91	0.90
24	1.2	1.63	1.94	1.17	1.11	0.89	1.75	65	1.0	1.43	2.05	1.06	1.29	1.26	0.83
25	8.7	0.93	1.03	1.43	1.02	1.86	1.28	66	4.8	1.04	0.20	0.30	0.67	0.65	0.20
26	6.2	0.82	0.55	0.70	1.17	0.41	0.66	67	57.7	1.15	0.41	0.38	1.04	1.05	12.00
27	26.3	0.79	0.73	1.41	0.85	1.88	2.33	68	58.9	0.76	0.85	2.00	1.59	1.23	0.66
28	0	0.40	0.92	1.48	1.83	1.45	0.42	69	3.4	0.89	0.82	0.83	1.30	0.98	1.75
29	0	3.35	0.53	0.27	0.78	1.08	3.83	70	4.6	1.56	2.78	1.25	1.07	1.52	1.21
30	33.4	0.49	0.77	0.68	0.53	0.42	0.30	71	8.0	1.82	1.73	1.66	0.78	0.69	0.41
31	0	2.36	1.28	0.59	0.59	0.85	2.57	72	1.0	1.55	1.59	1.59	1.36	1.46	2.80
32	0	2.20	3.16	2.78	2.95	2.50	0.55	73	4.5	0.88	2.04	1.47	1.08	0.91	0.55
33	0	0.50	0.42	0.53	0.32	0.38	0.60	74	19.5	0.91	0.92	0.78	1.12	1.37	0.69
34	4.0	0.85	3.26	1.01	1.61	1.27	2.66	75	5.2	0.50	0.42	0.78	0.70	0.54	0.47
35	33.9	0.06	0.57	2.91	0.40	0.12	0.12	76	10.5	0.94	1.31	1.64	1.66	1.43	1.90
36	0	10.75	0.18	0.96	0.92	3.39	8.50	77	0	0.91	0.17	0.74	0.56	0.72	1.00
37	0	0.95	1.45	1.63	4.17	3.01	1.00	78	31.5	1.04	4.11	1.45	1.12	1.17	0.61
38	0	1.20	0.69	0.41	0.50	0.77	1.70	79	1.0	1.32	1.17	0.80	1.48	1.40	1.07

Table 1. (continued)

Week	X	Ye	Yi	Yii	Yiii	Yiv	Yp	Week	X	Ye	Yi	Yii	Yiii	Yiv	Yp
39	3.0	1.31	1.91	0.40	0.72	0.45	0.20	80	4.0	0.92	0.99	1.12	1.00	0.85	1.57
40	34.7	1.66	2.30	2.00	1.01	0.90	0.66	81	0.1	0.99	0.86	0.69	0.61	0.92	0.45
41	72.0	0.40	1.86	0.37	0.55	0.43	1.25	82	13.8	0.80	1.39	0.85	0.78	1.05	0.60
42	11.0	2.67	0.81	5.40	2.53	3.62	2.00	83	2.0	0.61	0.98	1.10	0.82	0.33	1.16
43	8.4	1.34	1.60	0.84	0.89	0.80	0.60	84	1.5	2.13	1.55	1.40	1.73	4.27	4.71
44	0.8	0.65	0.63	2.06	1.47	1.97	2.00	85	0	1.17	0.44	0.49	0.49	0.63	0.57
45	0	0.51	0.44	0.15	0.24	0.21	0.25	86	24.5	0.44	1.81	2.00	1.79	1.29	1.26
46	11.5	0.80	4.40	0.82	1.10	0.61	0.66	87	0	1.90	0.69	0.61	0.64	0.87	2.16
47	21.0	2.90	0.66	4.36	3.56	2.79	3.50	88	92.8	0.50	1.07	1.45	1.25	1.27	0.53
48	24.2	0.66	0.60	0.67	1.28	1.58	1.14								

central whorl of leaf axils of a bromeliad. The procedure was repeated identically with instar IV larvae. We counted 20 eggs of *Wy. vanduzeei* into each of a number of vials and began adding the contents of the vials to the central whorl of leaf axils of the plants, then realized that we had insufficient eggs to treat each plant similarly (see table 2). Sufficient water was added that all leaf axils were almost full. Ten plants were placed outside the laboratory, under the canopy of an oak tree, as the first raindrops of the storm began falling; the other 10 plants were left in the laboratory. The storm was over by 5:15 p.m. and the 10 plants which had been placed outside were brought into the laboratory where all 20 plants were left there overnight. In the laboratory rain gauge 19.6 mm of rain were measured, all of this falling during the storm. The following morning the contents of the plants were removed using the apparatus described and illustrated by Frank et al. (1976) and the immature *Wy. vanduzeei* were counted (Table 2).

We had added 20 eggs to each plant. In no case did we recover all 20 eggs from any plant the following morning. Many of the eggs had hatched, but instar I larvae present were counted as if they were eggs. It is unlikely that eggs would have hatched during the brief period of the storm and all eggs and instar I larvae recovered were assumed to have spent the period of the storm as eggs. From the results of the analysis of field data we had expected loss of eggs from the plants. Calculation of a t-test ( $t_1 = -3.450$ ) indicated a highly significant difference ( $P < 0.005$  as a 1-tailed test) between number of eggs recovered from the control plants and number of eggs recovered from the plants placed out in the storm.

A few of the larvae we placed in the plants were recovered as pupae and it was evident that we could not differentiate between the instar III and the instar IV larvae we had placed in the plants. As a result of analysis of the field data, we had not expected loss of larvae from the plants, and this was confirmed in the experiment: the number of instar III and IV larvae and

Table 2. Eggs and larvae of *Wyeomyia vanduzeei* recovered from bromeliads after a thunderstorm. Before the thunderstorm 20 eggs, 10 instar III larvae and 10 instar IV larvae had been placed in each plant. The difference between numbers of eggs recovered from control plants and storm-exposed plants is significant ( $P < 0.005$ ). The difference between numbers of larvae recovered from control plants and storm-exposed plants is not significant.

Control plants			Plants in storm		
Plant no.	Eggs	Larvae	Plant no.	Eggs	Larvae
1	*	6	1	1	17
2	8	11	2	4	14
3	10	17	3	5	13
4	7	10	4	2	15
5	7	10	5	2	16
6	17	19	6	5	16
7	14	15	7	6	18
8	2	18	8	3	14
9	*	16	9	8	20
10	19	18	10	0	14

\* Indicates no eggs placed in this plant before storm (and none found after the storm).

pupae recovered from the control plants (140) was, in fact, slightly less than the number of individuals of the same instars recovered from the plants placed out in the storm (157), but with no significant difference. The plants had been hosed out adequately before the experiment since we recovered no instar II larvae from any plant.

Although from the field data we expected no loss of early instar larvae during heavy rain, we wished to confirm that this did not occur. Another opportunity arose on 13 August 1975 when another thunderstorm approached the area of the laboratory at 6:30 p.m. On this occasion 10 spare bromeliads of standard size were available and were hosed out quickly. We had instar I larvae in rearing dishes in the laboratory and 20 of these were placed in the central whorl of leaf axils of each of the 10 bromeliads. Water was then added to the leaf axils so that these were almost full. Five of the plants were placed under the tree canopy outside the laboratory in time to receive all the rainfall falling through the canopy as a result of the storm, during which 16.4 mm of rain were recorded. On this occasion the experimental plants were left out overnight, but the control plants

remained in the laboratory. The following morning the larvae present (all still as instar I) in each plant were extracted (Table 3). With a t-value of 0.739 there was clearly no significant difference between the number of larvae recovered from the control plants and storm-exposed plants.

CONCLUSION AND DISCUSSION. Our data show that eggs of *Wy. vanduzeei*, but not larvae, may be lost from bromeliads during heavy rainfall. These results are sufficiently conclusive, both from correlation

Table 3. Instar I larvae of *Wyeomyia vanduzeei* recovered from bromeliads after a thunderstorm. Before the storm 20 instar I larvae had been placed in each plant. The difference between numbers of larvae recovered from control plants and storm-exposed plants is not significant.

Control plants		Plants in storm	
Plant no.	Instar I larvae	Plant no.	Instar I larvae
1	14	1	12
2	19	2	15
3	17	3	17
4	13	4	18
5	18	5	13

analysis of field data and from direct field experimentation, to show that heavy rainfall must be considered to be an important mortality factor for eggs of *Wy. vanduzeei*. There can be no possibility of survival of eggs washed out of a bromeliad.

In the laboratory cages we have often observed adult female *Wy. vanduzeei* hovering over the central whorl of leaf axils of bromeliads, less frequently over outer leaf axils, and we have subsequently found eggs of *Wy. vanduzeei* in these bromeliads. Oviposition thus appears to occur while the female is in flight and the eggs appear to be dropped mainly into the central whorl. Eggs thus laid will float at the water surface unless stranded on the surfaces of leaves by falling water level. While floating eggs may be caused to move together and towards bromeliad leaves by the effects of surface tension, it is possible that stranded eggs may adhere to leaf surfaces by adhesion to organic substances including leaf exudates and, on further drying, may adhere quite firmly and may not be dislodged readily by rising water level. However, it is readily understandable how floating eggs may be flushed from a plant during heavy rainfall. It is also probable that floating eggs may be distributed from the central whorl to the other leaf axils by heavy rain.

Larvae and pupae of *Wy. vanduzeei* react to disturbance, in the same way as do larvae and pupae of other mosquitoes, by diving, and in this way must be able to avoid being flushed out of bromeliads.

Under the conditions of the 2 experiments in thunderstorms we recovered roughly 75% of *Wy. vanduzeei* larvae we

had placed in the plants and, although the data for comparison were inadequate, there appeared to be little difference in proportions recovered between instar I and combined instars III and IV. Since larvae seldom remain in the plants after use of our sampling methods, the loss of these larvae may be accounted for by one or more of the following explanations: (1) larvae died during the course of the experiment and were broken up by water pressure during the sampling process and forced through the 100-mesh stainless steel screen, (2) larvae were killed during the sampling process, broken up and forced through the screen, (3) larvae were overlooked among organic debris in petri dishes under the microscope and thus were not counted, (4) some larvae remained in the plants. The recovery of eggs was lower than that of larvae, but the same explanations may apply. It would be easier to overlook the presence of eggs than of larvae in petri dishes under the microscope because of the smaller size of the eggs and because of their immobility. Our estimates of numbers of eggs and larvae present in bromeliads in our study population are probably low and may have to be adjusted when adequate estimates of efficiency for our sampling apparatus are derived. However, present lack of such estimates does not affect the results here reported.

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