ON THE BIONOMICS OF BROMELIAD-INHABITING MOSQUITOES II. THE RELATIONSHIP OF BROMELIAD SIZE TO THE NUMBER OF IMMATURE *WYEOMYIA VANDUZEEI* AND *WY. MEDIOALBIPES*[1]

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ABSTRACT. In southern Florida the immature stages of the mosquitoes *Wyeomyia vanduzeei* Dyar and Knab and *Wy. medioalbipes* Lutz are found in water held in the leaf axils of bromeliads. As part of a study of the factors controlling mosquito population size we have examined the relationship between the size of bromeliads and the numbers of the immature stages of mosquitoes inhabiting them. Larger plants contain more mosquitoes, but the relationship differs in the different development stages of the insects and suggests density-dependent control of the numbers and rates of development of larvae.

INTRODUCTION

A natural population of the bromeliad *Tillandsia utriculata* L. includes plants of various sizes and of various physiological ages from minute newly-germinated plants to plants in flower and plants which, having produced seed, are dying or recently dead. Most leaf axils of the plants, except of the smallest plants and those which are dead or dying, trap rain water. Bromeliads of species which hold water in the leaf axils are known as tank bromeliads. In our study area near Vero Beach, Florida, *T. utriculata* is the only tank bromeliad present.

In southern Florida the immature stages of *Wyeomyia vanduzeei* Dyar and Knab and *Wy. medioalbipes* Lutz (we have arbitrarily followed Stone (1970) with regard to the name of this species which is eslewhere listed as *Wy. mitchellii* (Theobald)) are found in the water-filled leaf axils of *T. utriculata* and of other tank bromeliads. The number of immature mosquitoes present in the leaf axils of a bromeliad varies seasonally and from locality to locality; this, together with variances from plant to plant and sampling difficulties makes the estimation of numbers of immature mosquitoes within a bromeliad far from straightforward. Toward the goal of estimation of population size of *Wyeomyia* mosquitoes and of determination of the factors influencing population size, we have examined the relationship between the size of *T. utriculata* plants and the number of immature mosquitoes inhabiting them.

THE STUDY AREA. The predominant trees of our study area are white mangrove (*Laguncularia racemosa* (L.)) and buttonwood (*Conocarpus erectus* L.). Trees of both species support the growth of epiphytic bromeliads, but buttonwood, because of its rough bark, provides better anchorage for the epiphytes and maintains denser populations of these. In our initial survey of the area, we had found the average *T. utriculata* plant growing on a stem or branch of a tree at a height of about 140 cm from the ground.

We collected outside the study area a number of additional *T. utriculata* plants, whose leaf axils we hosed out to remove their contents. These were identified by plastic number tags, tied with nylon cord, and hung by wire S-hooks at a height of about 140 cm from the limbs of trees in the study area. Each of these plants was sampled on a given schedule according to its particular group. These additional plants were colonized by *Wyeomyia* mosquitoes from the existing population. Subsequently we removed from the area all the naturally occurring plants of size large enough to contain water in the leaf axils and thus able to support the immature stages of *Wyeomyia*. In this way we could readily sample all plants in the area, using

[1] Supported by NIH Research Grants Numbers AI–06587 and FR–05553.

the method described by Frank et al. (1976), for the immature stages of *Wyeomyia*; this method enabled us (loc. cit.) to demonstrate that plant size and presence of water in the leaf axils influence oviposition by *Wy. vanduzeei*, but that the presence of organic infusion and organic debris does not do so.

METHODS

We suspended in the study area 10 additional *T. utriculata* plants of each of 10 nominal size ranges, all of them capable of holding water in the leaf axils. Every plant was identified by a plastic number tag. Every week a plant of each of the 10 sizes was sampled and all of the contents of the leaf axils were removed before the plants were replaced, in the original position, in the study area. The random sampling sequence was arranged so that each plant was sampled once during a period of 10 weeks, then the same sequence was used thrice more for a total period of 40 weeks, extending from October 1974 to June 1975.

All of the immature stages of *Wy. vanduzeei* and of *Wy. medioalbipes* collected during sampling were identified and recorded and were not returned to the study area, but used in various laboratory observations. We measured the size of the plants at the onset of sampling in terms of the length of the longest leaf. At the end of sampling we again measured the length of the longest leaf and also measured the volumetric capacity of the leaf axils by filling each plant with water to its maximum holding capacity. The capacities of the plants ranged between 16 and 426 ml.

RESULTS

Numbers of immature *Wyeomyia* were recorded as numbers per plant, with a total of 40 replicates for plants of each size range. It was evident that *Wy. vanduzeei* outnumbered *Wy. medioalbipes* considerably. Scattergrams of numbers of individuals (y) vs. plant capacity (x) shown in Fig. 1, allowed the visual fitting of regression lines of less variance (than when using length of longest leaf as an indicator of plant size) and, believing the volumetric capacity to be in any case a better indicator of plant size, we have used it alone in the following calculations. Each data point in Fig. 1 (as also in Figs. 2, 5, 6 and 7) is the mean of 40 samples; the standard errors of the means are large not only because of the inter-plant variation, but also because of seasonal variation in mosquito population size. For the purposes of the analysis reported here the seasonal variation may be ignored, but it is to be analyzed in a separate publication.

WY. VANDUZEEI EGGS. The relationship between the number of *Wy. vanduzeei* eggs sampled (y) and plant capacity (x) was first demonstrated by correlation ($r = 0.9283$) to be significant ($P < 0.001$). The linear regression of form $y = \acute{a} + bx$, gave $\acute{a} = 0.2314$ and $b = 0.0340$. The value of b proved, of course, to be significantly different from zero. The origin of the regression line ($\acute{a} = 0.2314$) was slightly above the zero intercept of x and y, but so close that the biological meaning of the value of á was unclear. Conceivably oviposition could take place in plants of "less than" zero capacity (i.e. plants which are to some extent smaller than the minimum size necessary to hold water in the leaf axils) but which might be damp with moisture. We have demonstrated (Frank et al. 1976) that presence of water and plant size are factors influencing oviposition.

We performed an F-test to determine whether the calculated intercept is significantly different from zero and found ($F < 1$, $1/F = 19.74$) this not at all so. Therefore we were able to simplify the equation expressing the relationship of eggs to plant capacity, by eliminating the constant á, to $y = bx$, with $b = 0.0349$, which again was highly significant. (For method see Bliss, 1967.)

The scattergram with plotted regression line gave some slight visual indication that the true regression line might be curvilinear and sigmoidal (i.e. with 2 points of inflexion), shown in Fig. 2. Sigmoidal relationships are common in biological systems and there was no theoretical reason

why the observed relationship could not be of this form. We accepted the idea of an origin at (or very close to) the zero intercept and believed that any significant level of á would be most unlikely, so we calculated a cubic regression of form $y = c_1x + c_2x^2 + c_3x^3$, (where $c_1 = 2.32 \times 10^{-2}$, $c_2 = 1.28 \times 10^{-4}$, $c_3 = -2.58 \times 10^{-7}$) with the regression line constrained to pass through the zero intercept of x and y. This technique provided no improvement in fit ($F < 1$, $1/F = 1.01$) to the sample data.

The linear equation accepted, with b = 0.0349, necessarily also indicates that b = 0.0349 is the constant which describes the density of eggs (i.e. the number of eggs per ml of plant capacity). Thus, throughout the range of plant sizes sampled, the egg

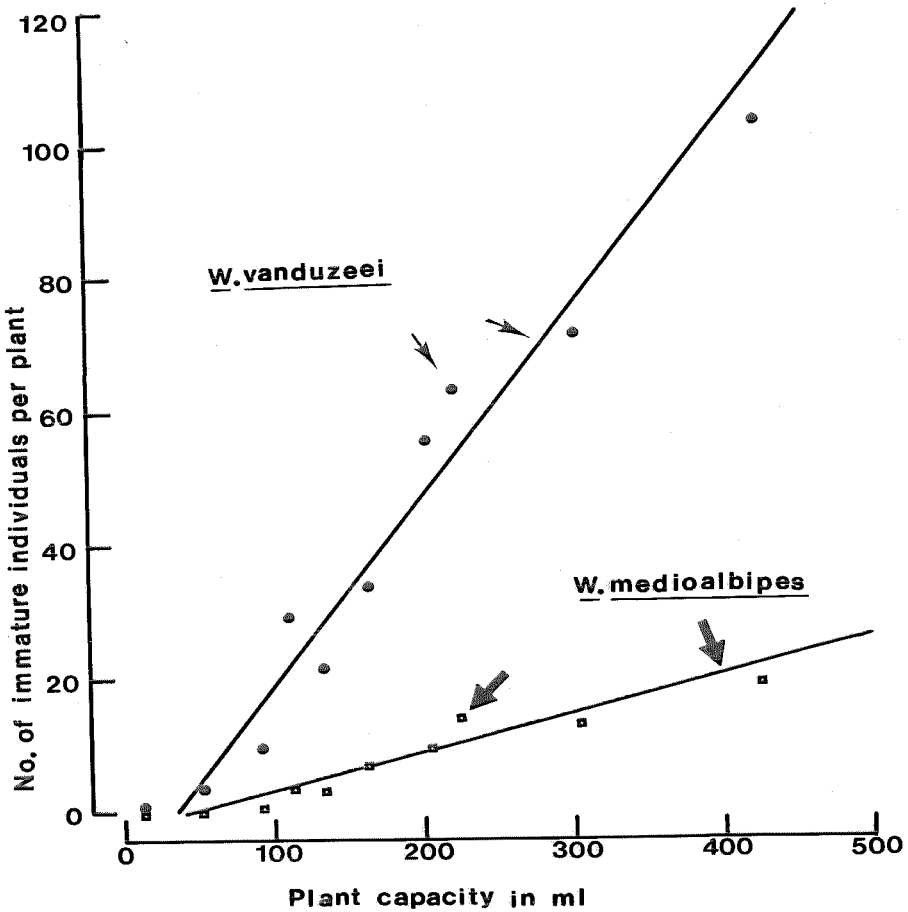

Fig. 1. Scattergrams of total no. (eggs + larvae + pupae) of *W. vanduzeei* and of *W. medioalbipes* vs. plant capacity in ml with fitted regression lines. *W. vanduzeei* outnumbers *W. medioalbipes*.

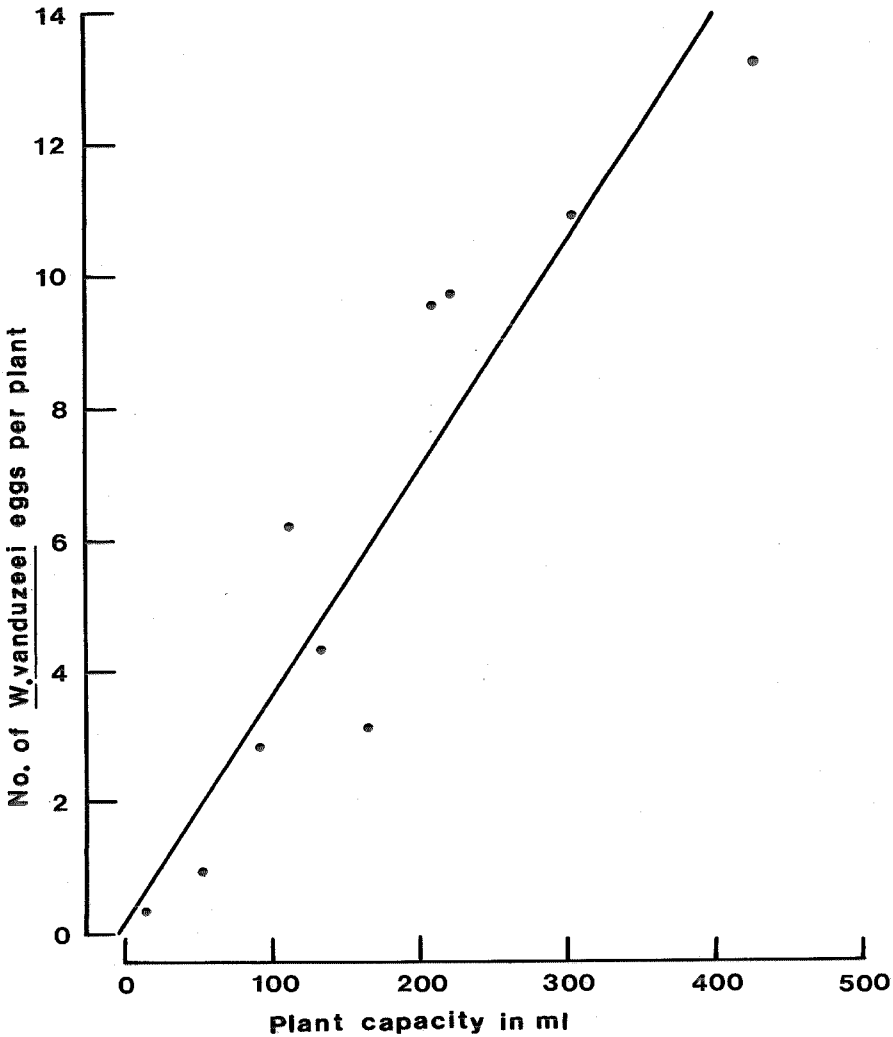

Fig. 2. Scattergram of no. of *W. vanduzeei* eggs vs. plant capacity in ml with fitted regression line y = bx where b = 0.0349

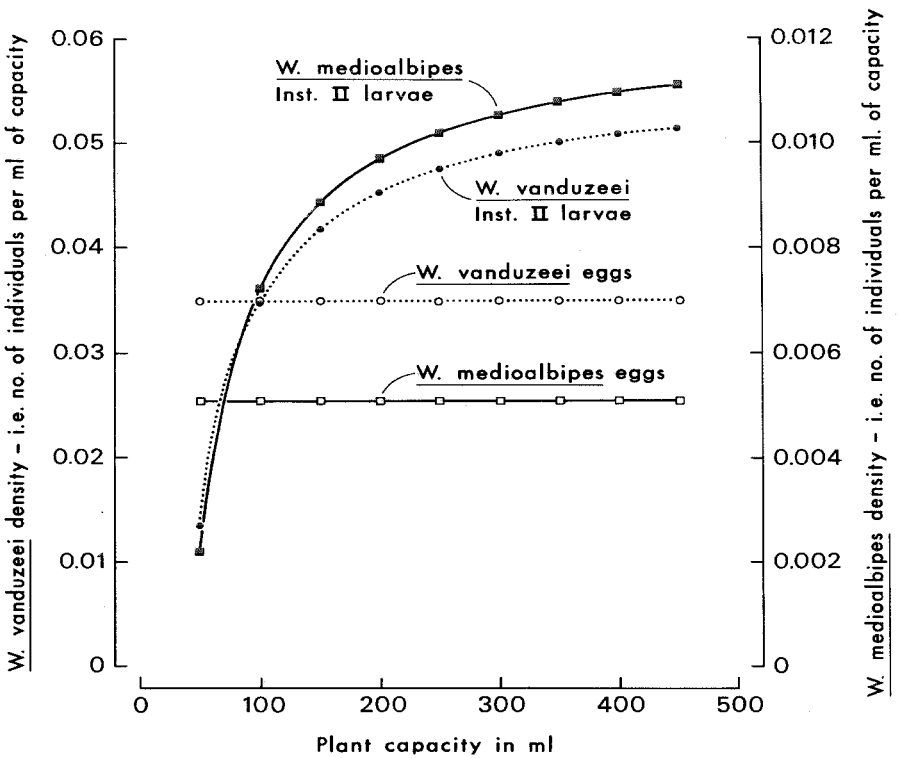

Fig. 3. Predicted densities of eggs and of instar II larvae of *W. vanduzeei* and *W. medioalbipes* (at different scales) vs. plant capacity in ml

density is calculated to be 0.0349 eggs per ml of plant capacity, shown in Fig. 3. The constancy of egg density was also verified by reference to the original data; when each value of y was divided by the appropriate value of x, and a scattergram of y/x vs. x drawn (Fig. 4), no trend could be discerned and the correlation of y/x vs. x was not significant ($P > 0.4$).

Wy. vanduzeei LARVAE. We carried out a similar procedure with the 4 larval instars of *Wy. vanduzeei*. All of them gave significant correlations ($P < 0.001$) (instar I: $r = 0.9786$, II: $r = 0.9875$, III: $r = 0.9761$, IV: $r = 0.9705$) of numbers of individuals vs. plant capacity. They differed from the eggs in that the calculated linear regressions all had negative values of á, not positive as with the eggs. Further, the x value (abscissa) with $y = 0$ for each instar fell in the range 24.7 to 38.0 ml, indicating that the linear equation would predict the complete absence of larvae in plants of less than 24.7 ml capacity. Because the sample data indicated the presence of some larvae of all instars in plants as small as 16.5 ml capacity, it was evident that the linear equation did not provide an adequate description of the sample data.

As with the eggs, we tested whether the intercept for each larval stage was significantly different from zero. All of the calcu-

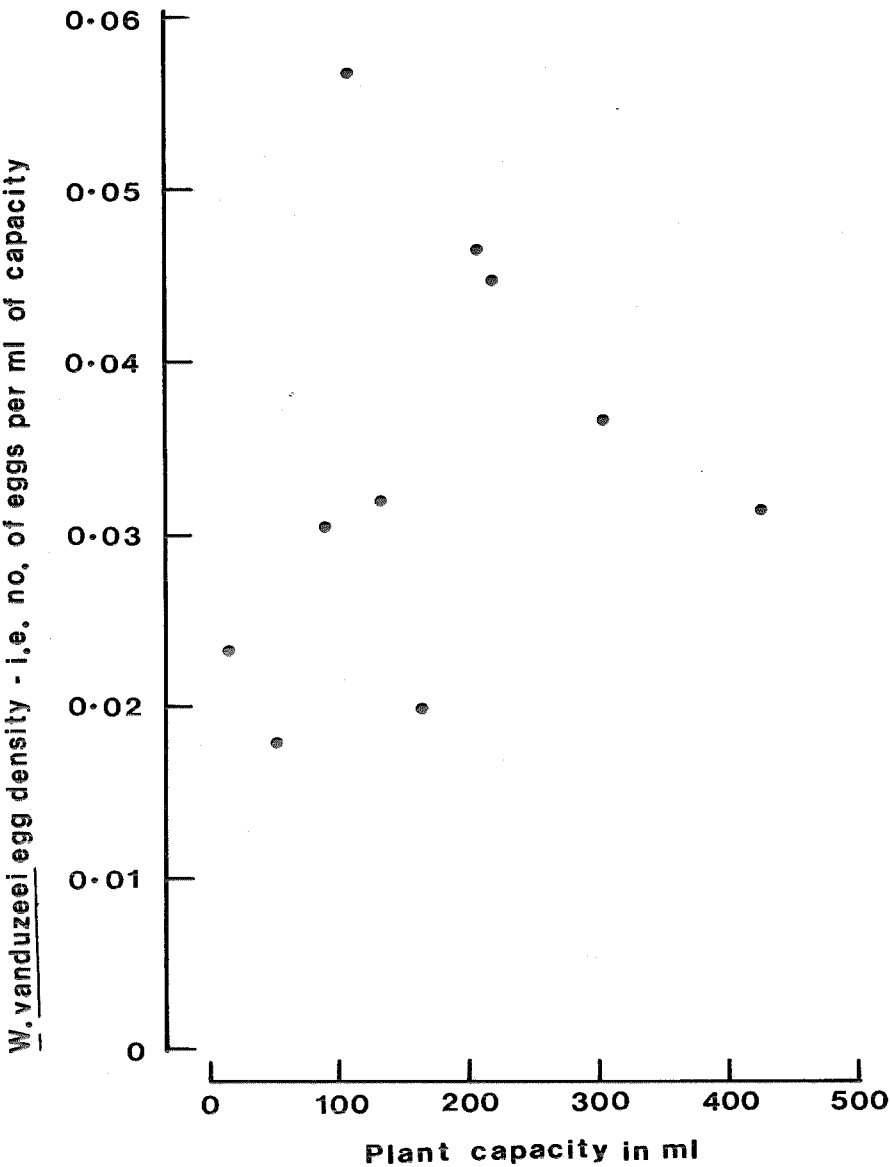

Fig. 4. Scattergram of density of *W. vanduzeei* eggs vs. plant capacity in ml

lated F-values were greater than unity (contrasting with the egg stage), but that for instar II larvae was the highest ($F = 10.33$, $P < 0.025$), with that for instar I larvae close to the 0.05 probability level, that for instar IV larvae close to the 0.10 probability level and that for instar II larvae exceeding the 0.25 probability level. Clearly the linear equation ($y = á + bx$) provides a significantly better fit to the sample data for instar II larvae (Fig. 5) than does the equation of form $y = bx$, it provides a better fit (with very little doubt) for instar I and IV larvae, and appears to provide a better fit for instar III larvae, although in this case there is some doubt.

As with the regression of number of eggs on plant capacity, more so with the larval instars, there was some indication that a curvilinear regression might fit the

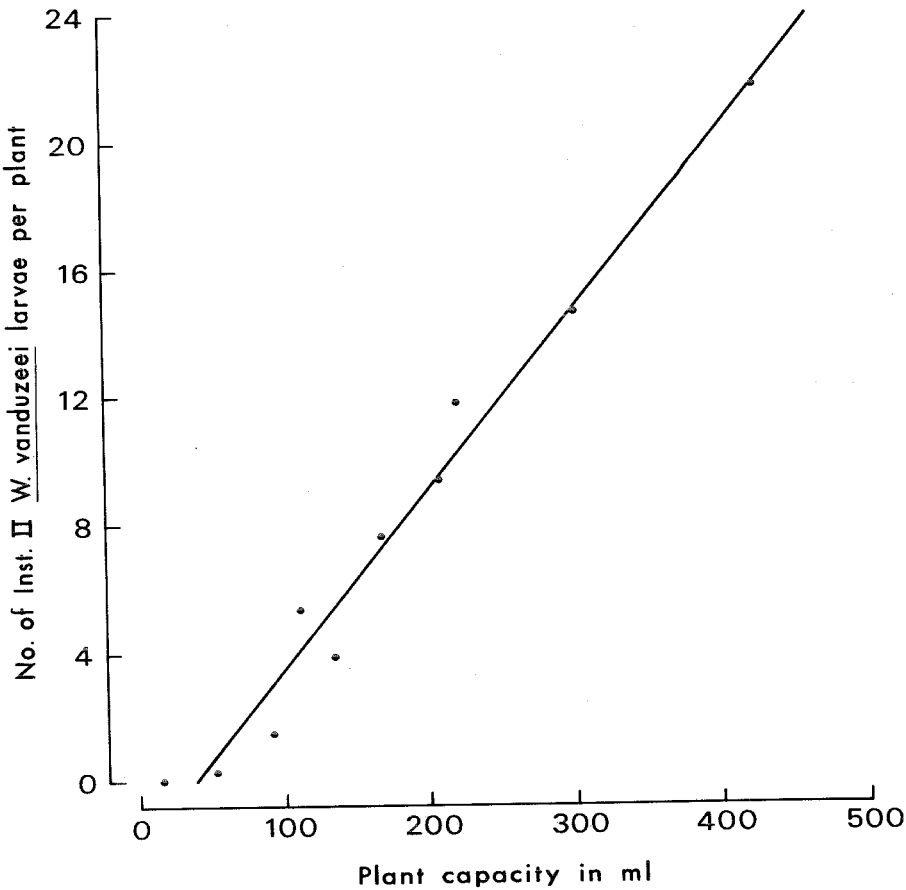

Fig. 5. Scattergram of no. of instar II *W. vanduzeei* larvae vs. plant capacity in ml with fitted regression line $y = á + bx$ where $á = -2.1295$ and $b = 0.0561$

sample data better than did the linear regression. Accordingly we calculated a quadratic regression ($y = q_1x + q_2x^2$) and a cubic regression ($y = c_1x + c_2x^2 + c_3x^3$) of instar IV larvae on plant capacity, and a quadratic, a cubic and a logarithmic ($\log y = á + bx$) regression of instar II larvae on plant capacity, but found that none of these gave any suggestion of an improved fit (allowing for the reduction in number of degrees of freedom) to the sample data. We have not calculated the cubic regression of instar III larvae on plant capacity but have relied upon visual examination of the scattergram in comparison with the scattergrams of instar II and IV larvae. From this we have concluded that a significantly better fit would not be obtained by the use of the cubic equation, the quadratic or the logarithmic, although the cubic would give some improvement in fit. We have therefore accepted the linear regressions of form $y = á + bx$ as providing the best available empirical fit to the sample data for all of the larval instars, even though we suspect some degree of curvilinearity in the regression line.

A theoretical argument should be presented here. If we accept totally that the regressions of instar I and II larvae are linear, then whatever the value of the abscissa at $y = 0$ for these instars must at least be equalled, if not exceeded by the value of the abscissa at $y = 0$ for instar III and IV larvae. In other words, if instar II larvae are not present in plants below a certain capacity, then instar III and IV larvae cannot be present in those plants. Therefore, if we accept that the regression of instar II larvae has an origin above and significantly different from zero, we must also accept that the regression of instar III and IV larvae is above and significantly different from zero. This suggests a single explanation: that the true regression lines are, in fact, curvilinear (and probably sigmoidal and probably with an origin at or very close to the zero intercept) but that there is too much variance in the sample data to allow for the significant fitting of a polynominal regression higher than the linear ($y = á + bx$). It should be noted that it is the instar III larvae whose numbers provide the greatest departure from the linear equation (Fig. 6) and which appear to have the greatest curvilinear component. The analysis of variance for the instar III larvae gave an F value of 161.618 ($P < 0.001$) indicating that the variance of the data points from the calculated linear regression is significant.

The calculated values for á and b for the 4 instars are as follows: instar I, á = −2.3721, b = 0.0707; instar II, á = −2.1295, b = 0.0561; instar III á = −1.4683, b = 0.0594; instar IV, á = −1.4279, b = 0.0450.

If we take the regression equation $y = á + bx$ and plot densities of larvae on plant capacity (as for instar II larvae in Fig. 3), using calculated values of y/x at increments of 50 ml, it is seen that density of the larvae increases asymptotically at increasing plant capacity. This is so for all the larval instars. If we were to assume that the true regression line of numbers on plant capacity is sigmoidal and on the basis of this to estimate values of y/x and plot them vs. x, we would arrive at a hyperbolic line in which density would at first increase, then decrease; however over the range of values of x considered (16.5 − 425.5 ml) the line would show an overall increase, even though at the higher values of x the values of y/x would be beginning to decrease. With both methods the density of all larval instars would be expected to increase in plants of capacities 0 to approximately 250 to 300 and thereafter might increase very slightly (linear equation) or might decrease (cubic equation). With both methods a positive correlation coefficient between larval density and plant capacity would be expected. Referring to our original data and dividing each value of y by the appropriate value of x, correlation coefficients of: instar I: 0.7020, instar II: 0.7662, instar III: 0.7009, instar IV: 0.6607 were obtained, all of which exceed the 0.05 probability level and are significant. For each instar a scattergram of y/x vs. y was drawn using original sample data and these demonstrated the asymptotic trend and also that in instar III larvae the

highest density values were reached in plants of average capacities 208.5 and 221.5 ml, thereafter declining slightly, with much less evidence of a subsequent decline in the other instars.

Increase in density of larvae in larger plants could be caused by (a) progressively delayed development of the larvae in larger plants, (b) progressively increased survival of the larvae in larger plants, or (c) a combination of both. It is noteworthy that in laboratory experiments with other genera of mosquito larvae, e.g. as reported by Wada (1965), high larval densities are associated with high larval mortality and prolongation of the larval development period. Our (incomplete and unpublished) laboratory trials with *Wyeomyia* species indicate that *Wyeomyia* larvae are not basically different in these respects.

Wy. vanduzeei PUPAE. Calculations of the number of *Wy. vanduzeei* pupae relative to plant capacity were performed in the same way as for eggs and larvae. A correlation coefficient of 0.9074 was obtained. The regression equation of form $y = \acute{a} + bx$ did not provide a significantly better fit to the sample data than did the equation of form

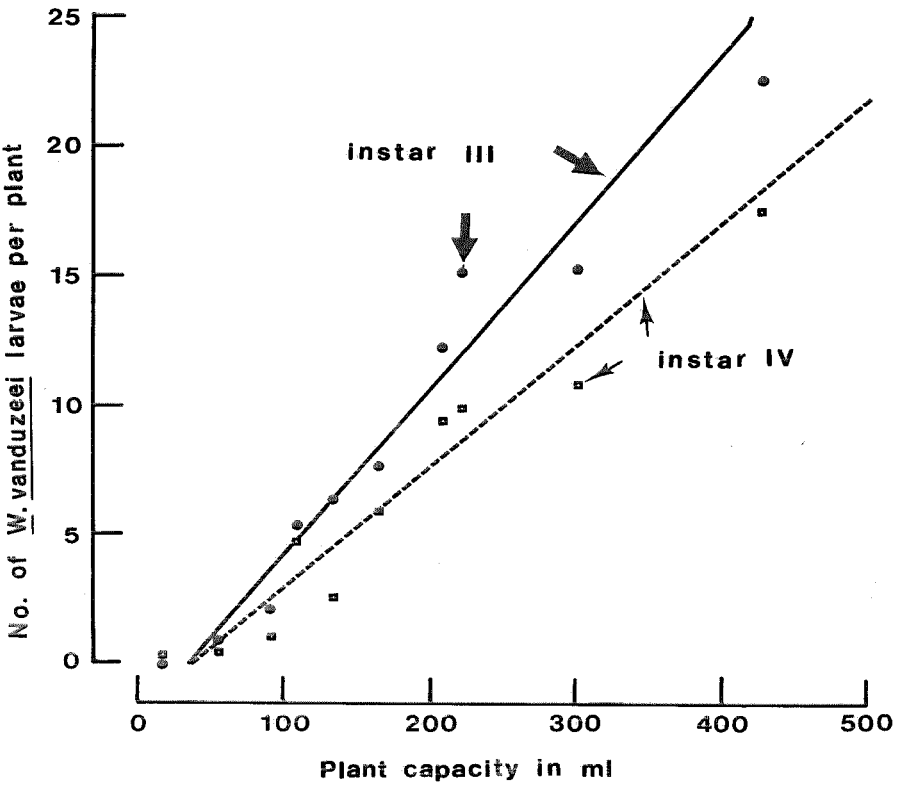

Fig. 6. Scattergrams of no. of instar III and IV *W. vanduzeei* larvae vs. plant capacity in ml with fitted regression lines $y = \acute{a} + bx$ where $\acute{a} = -1.4683$ (inst. III) -1.4279 (inst. IV) and $b = 0.0594$ (inst. III) 0.0450 (inst. IV).

$y = bx$. With the former equation the calculated abscissa of $x = 28.6$ at $y = 0$ falls in the same range (24.7 to 38.0) as noted with the larvae, and the value of á is, correspondingly, negative.

Visual examination of the scattergram of numbers of pupae vs. plant capacity (Fig. 7) indicated more variance of the observed data points about the calculated line than with any larval instar, but yet no more evidence that a quadratic or cubic equation would provide a line of better fit. We attribute the larger variance to the much smaller number of pupae, than of eggs or of any larval instar, taken from plants sampled.

Because, apparently, the equation $y = bx$ provides the best empirical fit to the sample data, we have no significant evidence that the density of pupae increases at higher plant capacities. Additionally, the correlation of pupal density with plant capacity (using original data) gave an insignificant coefficient of 0.3438 ($P < 0.4$).

The only evidence we have for increased pupal density at higher plant capacities comes from the calculated value of the abscissa at $y = 0$ (with equation $y = á + bx$) falling in the same range as with the larvae. This apparent similarity would arguably make it possible to view the true regression line of pupal numbers on plant capacity in the same way as larval numbers on plant capacity, as sigmoidal.

Wy. medioalbipes EGGS. Correlation coefficients of numbers of *Wy. medioalbipes* eggs, all larval instars and pupae on plant capacity were all highly significant and, as with all stages of *Wy. vanduzeei*, exceeded the 0.001 probability level.

Linear regressions of the number of eggs (y) on plant capacity (x) using equations $y = á + bx$ and $y = bx$ demonstrated that with the former equation, the value of á is slightly positive as with *Wy. vanduzeei* eggs. Also, as with *Wy. vanduzeei* eggs, the intercept of the regression line gave no indication of being significantly different from zero. The scattergram with calculated linear regression line gave no visual indication of curvilinearity. From the original data, the values of y (number of eggs) divided by x (appropriate plant capacity) to give density, plotted on plant capacity, gave no indication of any trend nor any significant correlation ($r = -0.3651$).

Accordingly we accept the regression equation $y = bx$, with the value of $b = 0.00508$ as also representing the constant (Fig. 3) which describes the density of eggs at all plant capacities.

Wy. medioalbipes LARVAE. Linear regressions of the numbers of larvae (y) (each instar separately) on plant capacity (x) using equations $y = á + bx$ and $y = bx$ demonstrated that with the former equation the value of á is negative, as with *Wy. vanduzeei* larvae. The value of the abscissa with $y = 0$ for instar I, II and IV larvae was in the range 34 to 43 ml, i.e. slightly higher than with *Wy. vanduzeei* larvae. For instar I, II and IV larvae, F-values for testing the significance of the intercept were lower than with *Wy. vanduzeei* larvae; all exceeded the 0.25 probability level, but only that for instar I larvae exceeded the 0.10 probability level. Accordingly, less confidence may be attached to the values of the intercepts and consequently to the slopes of the regression lines. The scattergrams gave slightly more visual evidence of curvilinearity (sigmoidal) than those of the same instars of *Wy. vanduzeei*, but still insufficient to justify computation of the cubic equation. Consideration of the original data and calculation of correlation coefficients of larval density on plant capacity, revealed that all three coefficients exceeded the 0.10 probability level, while those for instar I and IV larvae exceeded the 0.02 probability level. Since density of these larval instars is evidently not constant, the formula $y = bx$ does not adequately describe the relationship of their numbers to plant capacity. Examination of the scattergrams of larval density on plant capacity (from original data) revealed evidence in all three instars that the trend diverged from the asymptotic to indicate a reduction in larval density in the largest plants, in contrast to the general increase in density with plant capacity in most of the plants.

In the instar III larvae alone the regres-

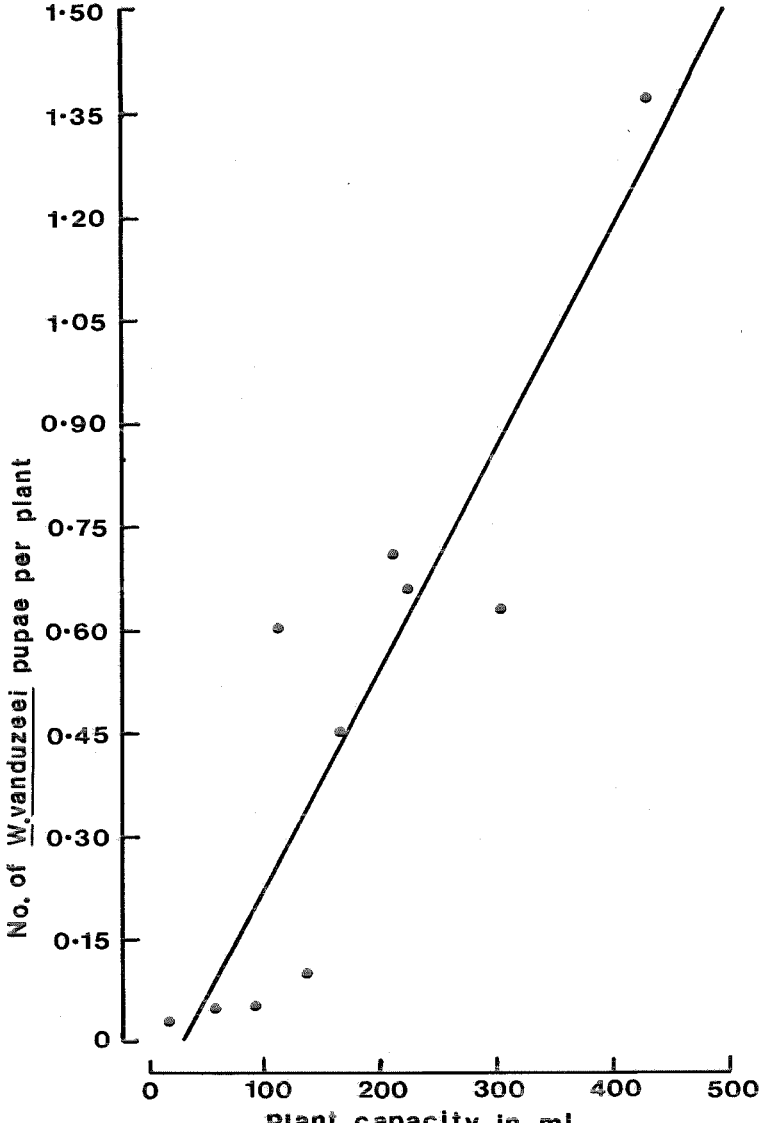

Fig. 7. Scattergram of no. of *W. vanduzeei* pupae vs. plant capacity in ml with fitted regression line $y = á + bx$ where $á = -0.0846$ and $b = 0.0032$

sion of larval numbers on plant capacity using the cubic equation ($y = c_1x + c_2x^2 + c_3x^3$) indicated that this gave a better fit to the sample data ($F = 3.7$, $P < 0.10$) than did the linear equation ($y = á + bx$). The calculated constants were $c_1 = 6.301 \times 10^{-4}$, $c_2 = 6.947 \times 10^{-5}$, $c_3 = -1.262 \times 10^{-7}$. It seems that the regression of instar III *Wy. medioalbipes* larvae has a more evident curvilinear component than has any of the regressions for other stages of either species. This parallels the observation that the regression of *Wy. vanduzeei* instar III larvae on plant capacity appears to be more sigmoidal than that of any other stage of *Wy. vanduzeei*.

The cubic regression for *Wy. medioalbipes* instar III larvae is almost a straight line over the greater part of its length, being curved only at its extremes and, if we extrapolate the straight part of the regression line we find that the value of the abscissa with $y = 0$ would fall in the same range (34 to 43 ml) as indicated by the linear regressions of the other larval instars of *Wy. medoalbipes*. The scattergram of the density of instar III larvae on plant capacity (from original data) reveals a reduction in larval density in the largest plants (as opposed to the general trend) and, of course, this trend agrees well with the calculated values of larval density on plant capacity derived from the cubic regression equation. The correlation ($P < 0.10$) of larval density on increase in plant capacity, based on original data, was one of the least significant for any larval instar of either species, but this is to be expected in view of the more pronounced reduction in larval density at the largest plant capacities.

Wy. medioalbipes PUPAE. The correlation coefficient of the number of pupae vs. plant capacity was significant ($P < 0.001$), but pupae were absent from 4 of the 5 smallest sizes of plants. This absence made the regression analysis of the smaller plant capacities theoretical, and has prevented us from drawing any valid conclusions from our data.

SUMMARY AND DISCUSSION

There were significantly more eggs of both *Wy. vanduzeei* and *Wy. medioalbipes* in larger plants. The number of eggs sampled was closely proportional to the volumetric capacity of the plants, so that egg density was assumed to be constant in both species (Fig. 3).

With *Wy. vanduzeei* and *Wy. medioalbipes* larvae there were also significantly more larvae in larger plants, but the number of larvae was not directly proportional to the volumetric capacity of the plants, shown for *W. vanduzeei* instar II larvae in Fig. 2. Density of all 4 instars of both species increased approximately asymptotically with increasing plant capacity, as indicated for instar II larvae of both species in Fig. 3. There is evidence for believing that in all larvae, more so in *Wy. medioalbipes* and especially in instar III larvae, there is some decrease in density in the largest plants.

With both *Wy. vanduzeei* and *Wy. medioalbipes* pupae there were significantly more pupae in the larger plants. In both species the density could not be demonstrated to be other than constant in plants of various sizes.

The measured increase in larval density in larger plants must be attributed to (a) progressively delayed development of the larvae in larger plants, (b) progressively increased survival between the egg stage and the appropriate larval stage in larger plants, or (c) a combination of both. Substantial evidence of an increase in pupal density in larger plants, in the absence of an increase in egg density, would have implied increased survival between the egg stage and the pupal stage, because the development times of these stages are influenced only by temperature (provided they are in water) in most mosquitoes (Nielsen and Evans 1960; Reiter and Jones 1975) and probably so in *Wyeomyia* (our unpublished laboratory data do not indicate otherwise). If there is an increased survival between the egg and larval stages in larger plants, this is not readily apparent in in-

creased survival between the egg and pupal stage, therefore at least in later instars, much of the apparent increase in density must be attributed to delayed development, to which increased survival is the key. In other words, with some increase in survival (perhaps limited to earlier instars, perhaps not), density of larvae increases; when density increases this leads to delayed development which causes an apparent further increase in density. This situation cannot continue to amplify without there being a reduction in survival. The reduction in survival may be indicated by the levelling out of the asymptotic curves (as in Fig. 3), or even in the reduction in density in the largest plants which is most strongly evident in instar III larvae. It may thus be that the greatest delay in development is to be found in instar III larvae.

ACKNOWLEDGMENT

We wish to thank Dr. Terry Rickard of the ORINCON Corp. for his critical reading and comments on the statistical aspects of this paper.

References Cited

Bliss, C. I. 1967. Statistics in biology. Statistical methods for research in the natural sciences. Vol. I. New York, McGraw-Hill, XIV + 558 pp.

Frank, J. H., Curtis, G. A. and Evans, H. T. 1976. On the bionomics of bromeliad-inhabiting mosquitoes. I. Some factors influencing oviposition by *Wyeomyia vanduzeei*. Mosquito News 36 (1): 25–30.

Nielsen, E. T. and Evans, D. G. 1960. Duration of the pupal stage of *Aedes taeniorhynchus* with a discussion of the velocity of development as a function of temperature. Acta Oecol. Scand. 11:200–222.

Reiter, P. and Jones, M. D. R. 1975. An eclosion timing mechanism in the mosquito *Anopheles gambiae*. J. Entomol. (A) 50:161–168.

Stone, A. 1970. A synoptic catalog of the mosquitoes of the world, supplement IV. (Diptera, Culicidae). Proc. Entomol. Soc. Wash. 72:137–171.

Wada, V. 1965. Effects of larval density on the development of *Aedes aegypti* (L.) and the size of adults. Quaest. Entomol. 1:223–249.

LABORATORY RESISTANCE OF THE MOSQUITO *ANOPHELES QUADRIMACULATUS* TO THE MERMITHID NEMATODE *DIXIMERMIS PETERSENI*

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ABSTRACT. Larvae of a Lake Charles colony of *Anopheles quadrimaculatus* that has been used for 4 years to produce *Diximermis peterseni* now show resistance to this biological agent. The infection rate by the nematode is only half that of the Gainesville colony from which the Lake Charles colony originated. The mechanism of resistance was observed to be avoidance of attack by larvae when nematode larvae were present.

In the course of routine culturing of the mermithid nematode *Diximermis peterseni* in the mosquito host *Anopheles quadrimaculatus* at the Gulf Coast Mosquito Research Laboratory, Lake Charles, Louisiana, we observed a decline in nematode production. This laboratory colony, which had been used the past 4 years to produce *D. peterseni* (a possible biological agent) was obtained from the Insects Affecting Man Research Laboratory, Agricultural Research Service, U.S.D.A., Gainesville, Florida, about 12 years ago. The culture method was as fol-