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MOSQUITO LARVAE IN AXILS OF THE IMPORTED BROMELIAD *BILLBERGIA PYRAMIDALIS* IN SOUTHERN FLORIDA

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ABSTRACT

At monthly intervals for a year, in Daytona, Tampa, Vero Beach and Miami, a survey was conducted of the aquatic stages of mosquitoes existing in water impounded by the leaves of the imported bromeliad *Billbergia pyramidalis*. A few *Aedes aegypti*, *Culex quinquefasciatus*, *Toxorhynchites rutilus* and *Corethrella appendiculata* were found, but almost all of the mosquitoes belonged to the genus *Wyeomyia*, *Wyeomyia vanduzeei* was predominant at 3 of 5 sites in Miami, but at all the other sites *W. mitchellii* was predominant. Average annual production of *Wyeomyia* per bromeliad was estimated as 107 adults based upon the number of pupae collected. Pupae were found throughout the year. There was a linear relationship of numbers of pupae to numbers of eggs + larvae collected at lower densities of eggs + larvae.

RESUMEN

En las ciudades de Daytona, Tampa, Vero Beach y Miami se conducieron encuestas todos los meses durante un año de las etapas acuáticas de los mosquitos que existen en el agua embalsada por las hojas de la bromelia importada *Billbergia pyramidalis*. Unos cuantos *Aedes aegypti*, *Culex quinquefasciatus*, *Toxorhynchites rutilus* y *Corethrella appendiculata* fueron encontrados, pero casi todos los mosquitos pertenecían al género *Wyeomyia*. *Wyeomyia vanduzeei* fue el mas abundante en 3 de los 5 sitios en Miami, pero en todos los demás sitios, la especie predominante fue *W. mitchellii*. La producción media anual de *Wyeomyia* fue estimada a ser 107 adultos, basado en el

número de pupas recobradas. Pupas fueron encontradas durante todo el año. Existe una relación directa entre el número de pupas y el numero de huevos + larvas que fueron coleccionadas en densidades bajas de huevos + larvas.

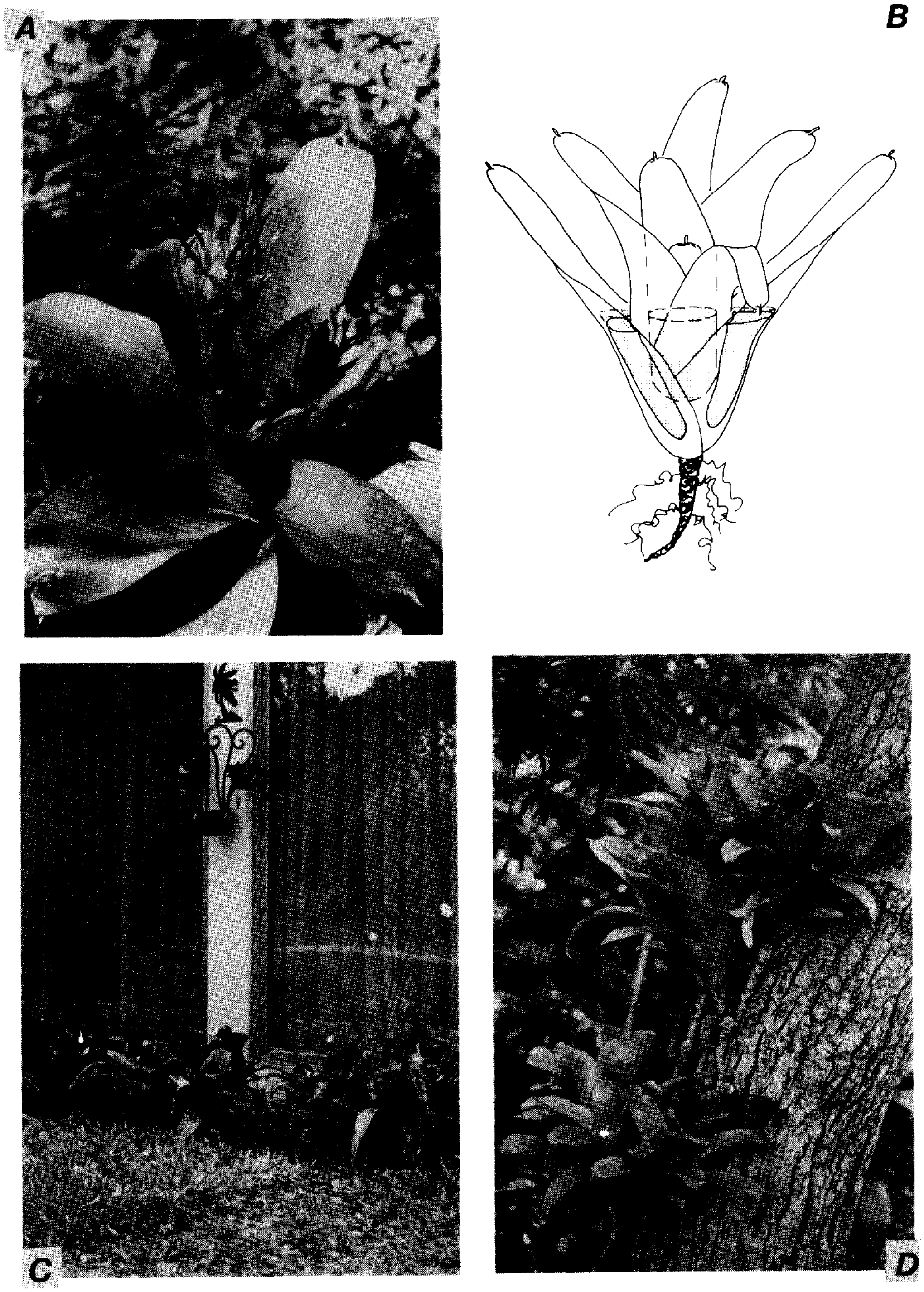

Fig. 1. *Billbergia pyramidalis*, (a) in flower, (b) showing water-impounding capacity, (c) growing in an urban habitat in Florida, (d) growing epiphytically in Florida.

Billbergia pyramidalis (Sims) Lindley (Fig. 1) is a Brazilian bromeliad which is cultivated widely as an ornamental (Padilla 1973). Southern Florida's climate is sufficiently frost-free for the plant to be grown outdoors in many cities. All bromeliads native to Florida are epiphytic, but *B. pyramidalis* grows on the ground as well. It reproduces vegetatively, needs no maintenance when cultivated in tree-shaded habitats, and will form dense beds if left unattended. It is probably the most abundant introduced bromeliad in southern Florida.

Many bromeliads impound water in their leaf axils, forming a reservoir as a source of nutrients. Although we are not aware of studies showing that *B. pyramidalis* absorbs nutrients from the reservoir, its ability to grow epiphytically suggests that it uses this method of nutrient procurement. The central whorl of leaves forms a cup with a few peripheral water-impounding axils (Frank & O'Meara 1984). Questions from mosquito control personnel about the incidence of *Aedes aegypti* (L.) larvae in bromeliad leaf axils prompted a survey of mosquito larvae in *B. pyramidalis* reservoirs in 4 cities in southern Florida. The objectives were to rank the incidence of the mosquito species encountered, to explain the ranking, and to estimate production of adult mosquitoes. To obtain a geographically and seasonally representative sample, and for logistic reasons, samples were taken monthly for a year in Daytona, Tampa, Vero Beach, and Miami (Fig. 2).

MATERIALS AND METHODS

Sample size and methods

An oak woodland at the western boundary of Florida Medical Entomology Laboratory, Vero Beach, was planted with hundreds of *B. pyramidalis* in 1976-1977. In April 1978, 10 dispersed, large plants without flowers were selected for sampling. They were uprooted without spilling the reservoir contents, and leaf litter was removed by forceps from the reservoir. The cup of the reservoir of each plant was filled with 30-50 ml of tapwater. A large glass syringe (sold as a meat baster) was used to transfer as much water as possible from the cup into a 500-ml container, keeping the fluid removed from each plant separate. This fluid, in every case, measured less than 100 ml. The contents of each container were examined under a dissecting microscope and all mosquito larvae and pupae were picked out, counted, and recorded.

Each plant was then washed out thoroughly using a sampling apparatus described by Frank et al. (1976). The washings from each plant were placed into separate containers which also were examined for mosquito larvae and pupae as above. These methods provided for mosquito larvae and pupae (a) a mean ± SD (31.4 ± 33.3) for numbers extracted by syringe from the cups of the 10 plants, and (b) an estimate of the numbers in the cups as a percentage of the numbers in the entire reservoir including peripheral axils (38%). It was thus found that a glass syringe could be used to sample the contents of the cup of the reservoir and thereby to estimate the numbers of mosquito larvae and pupae per plant (forcing the syringe into the peripheral axils damaged the plant). Necessary sample size to determine the mean ± 10 with 95% confidence was calculated as $4 \times 33.3^2/10^2 = 45$ plants (equation from Poole 1974).

Sample localities

Monthly sampling from *B. pyramidalis* was begun in the first full week of May 1978 at the same Vero Beach site. The procedure described above with a glass syringe and added water was used to extract the liquid content of the central cup of 45 plants into 500 ml plastic containers for immediate transport to the laboratory. There, contents of the plastic containers were washed into 45 100-ml petri dishes for microscopic examination. All mosquito larvae and pupae were removed by dropper from the petri dishes;

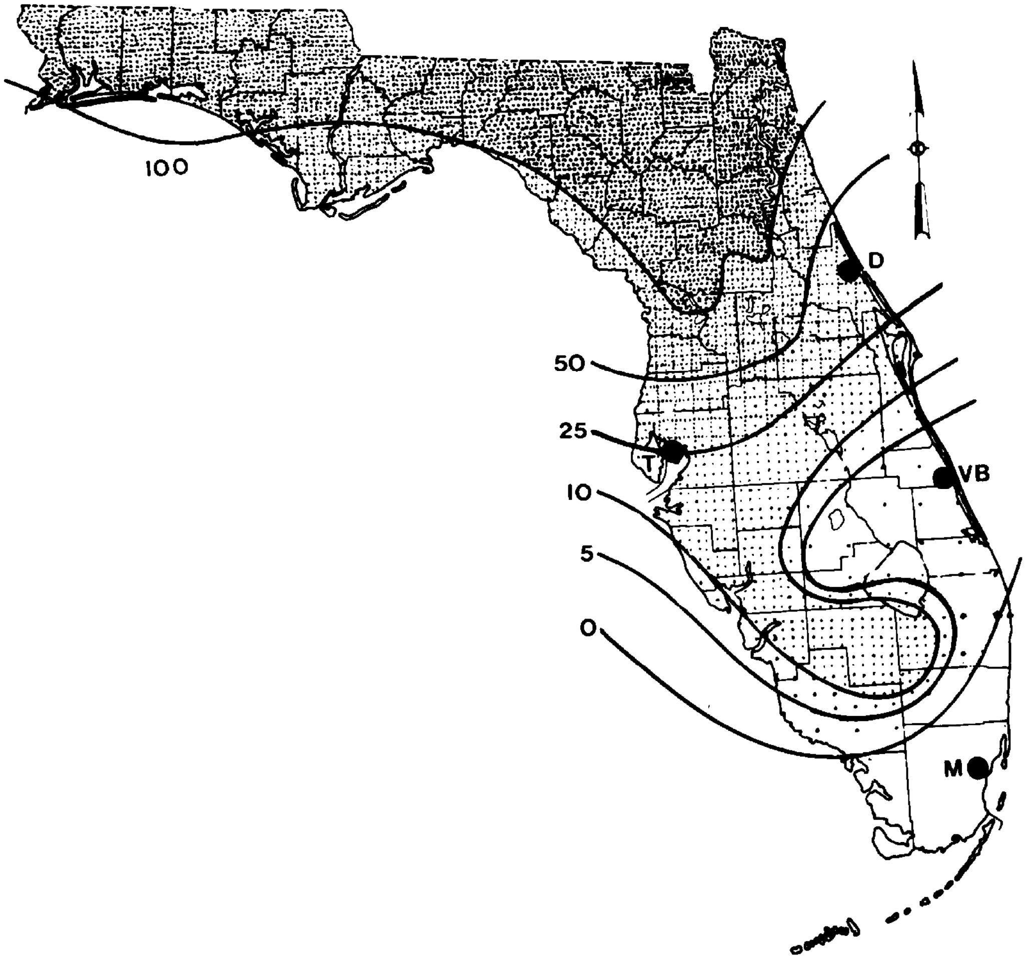

Fig. 2. Map of Florida showing isothermal lines and location of the 4 cities (D: Daytona, T: Tampa; V: Vero Beach, M: Miami) where collections were made. Isothermal lines show the average annual no. of freezing hours (after Raisz 1964).

the dishes were left to stand for a week to allow hatching of eggs present, then the resultant larvae were removed, identified and recorded. Larvae and pupae recovered on the day of sampling were then identified, counted and recorded; instar I larvae which could not be identified specifically were placed into small plastic petri dishes, allowed to develop to instar II, then identified. A large leaf of each bromeliad sampled was marked by paper-punch to give a numerical code of holes to ensure that no bromeliad was sampled in successive months. Monthly sampling was ended after the first full week in July 1979.

The same procedure was begun at Miami in the first full week of June 1978 with the following differences. Five urban sites were selected and 9 *B. pyramidalis* were sampled at each site. Water in 4-liter plastic containers was carried to the field where samples from the cups of the bromeliads were extracted into plastic bags which were labelled, sealed, and placed in a cool chest for transport the same day to Vero Beach; they were examined the next day. Starting in October 1978, samples were shipped to Vero Beach on the day after collection and were examined the next day.

The same procedure used at Miami was adopted at Tampa, beginning in the first full week of July 1978, with the difference that samples were collected into 500-ml plastic containers and transported by car to Vero Beach on the day of sampling. Thus, samples from Tampa were examined the day after collection.

A slightly different procedure was used at Daytona. Five *B. pyramidalis* were sampled at each of 9 urban sites and the contents of the cups of the plants were immediately preserved in alcohol until time could be found for examination by personnel of East Volusia Mosquito Control District. Mosquito eggs and instar I larvae were difficult to find and identify, so were not recorded.

Almost all preimaginal mosquitoes from Vero Beach, Miami and Tampa were examined alive; very few specimens were discovered dead. Movement of these living specimens in water in petri dishes made detection easy under the microscope. In contrast, those from Daytona were preserved in alcohol. Instar II larvae were recorded in very small numbers from Daytona relative to those collected elsewhere, and we suspect that many were overlooked among the alcohol-preserved debris from the bromeliad cups.

Bromeliads at the Vero Beach site grew under homogeneous conditions; estimated means from the 45 bromeliads sampled there monthly should fall within 10 of the true mean. This was not true of the deliberately dispersed sites at the other cities (most sites were in the yards of houses, but one each at Miami and Tampa were in parks) where the only criterion was that an adequate number of *B. pyramidalis* should exist with at least partial shading by trees. Samples from Vero Beach were thus the basis for comparisons.

RESULTS AND DISCUSSION

Data for 12 months (August 1978-July 1979) showed the most abundant preimaginal mosquitoes were *Wyeomyia* (Table 1). *Wyeomyia mitchellii* (Theobald) was the most abundant species at the 3 more northerly cities, but *Wyeomyia vanduzeei* Dyar & Knab was more abundant in Miami. However, whereas *W. mitchellii* was the more abundant species at all sites in the 3 northerly cities, it was more abundant than *W. vanduzeei* at 2 of the 5 Miami sites [sites 1 and 2 which were east of the other 3 and apparently (we have no exact measurements) more deeply shaded]. The other 4 species (*Culex quinquefasciatus* Say, *Aedes aegypti* (L.), *Toxorhynchites rutilus* (Coquillett) and *Corethrella appendiculata* Grabham) were uncommon, and the first of them was associated with putrid water caused by grass clippings ejected into *B. pyramidalis* reservoirs.

TABLE 1. AQUATIC STAGES OF MOSQUITOES COLLECTED FROM 45 *BILLBERGIA PYRAMIDALIS* PLANTS IN 12 MONTHLY SAMPLES AT 4 CITIES IN FLORIDA.

	Daytona	Tampa	Vero Beach	Miami
Wyeomyia mitchellii	1,550	4,097	19,759	1,497
Wyeomyia vanduzeei	85	43	2,052	3,571
Aede aegypti	48	16	5	100
Culex quinquefasciatus	29	85	0	118
Toxorhynchites rutilus	1	3	0	4
Corethrella appendiculata	0	0	0	8

Numbers for Daytona include only instar III-IV larvae and pupae, whereas numbers for other cities include also instar I-II larvae and eggs; see text for explanation.

TABLE 2. *WYEOMYIA* PUPAE COLLECTED FROM 45 *BILLBERGIA PYRAMIDALIS* PLANTS IN 12 MONTHLY SAMPLES AT 4 CITIES, AND ESTIMATED ADULT PRODUCTION PER PLANT PER YEAR. SEE TEXT FOR METHOD OF ESTIMATION.

	Number sampled		Est. annual production	
	mitchellii	*vanduzeei*	*mitchellii*	*vanduzeei*
Daytona	214	18	85	7
Tampa	180	0	71	0
Vero Beach	318	52	126	21
Miami	78	222	31	88

Mosquito production

Production of adult *Wyeomyia* per bromeliad per year was estimated from the numbers of pupae collected (Table 2). Each estimate was calculated as (100/38)365N/(12 X 4.5 X 45) and assumed the development time of each pupa averaged 4.5 days (Frank unpubl.), where N is the no. of pupae collected from 45 bromeliads in 12 visits during a year of 365 days, and that 38% of the pupae present were collected. The estimate assumed that all pupae collected would have survived to produce adults. Estimated production averaged 107 adults/bromeliad/year and ranged from 71 (Tampa) to 147 (Vero Beach), only about a twofold difference. Some of the sites contained hundreds of *B. pyramidalis*.

Species composition

Aquatic stages of *W. vanduzeei* and *W. mitchellii* have rarely been recorded in Florida from habitats other than bromeliad leaf axils. The northern limits of distribution for both species are approximately Volusia County on the east coast and Hillsborough or Pasco County on the west coast (Breeland 1982), at about the 50 hr isothermal line (Fig. 2), which is the approximate northern limit of indigenous water-impounding bromeliads of the genus *Tillandsia*. Despite the predominance of *W. vanduzeei* in Miami but of *W. mitchellii* at the 3 more northern cities, there is yet no evidence that *W. vanduzeei* is more restricted by low winter temperatures. Instead, it has been found that *W. mitchellii* adults are more restricted to tree-shaded habitats, whereas *W. vanduzeei* is more prone to fly out into unshaded areas (Frank & O'Meara 1985).

Variation in dominance of the 2 *Wyeomyia* species among sites in Miami prompted a G-test to examine the interaction between species (2), time (12), and site (5). Species composition was dependent on both time (G = 173, df = 11, $P > 0.001$) and site (G = 2,133, df = 4, $P << 0.001$), but site was a much more important determinant as shown by the much higher G-value. Lowest numbers of both species occurred in Miami in March; highest numbers of *W. vanduzeei* occurred in November, very closely followed by June, whereas highest numbers of *W. mitchellii* occurred in June, very closely followed by November. In short, the seasonality of abundance of the two species appeared very similar.

The 4 other mosquito species encountered were uncommon. *Aedes aegypti*, *C. quinquefasciatus* and *T. rutilus* females prefer to oviposit in darkly colored containers as compared with the light green of bromeliads (Frank 1985). It is probable that *C. appendiculata*, whose aquatic stages typically inhabit treeholes, prefers dark ovipositional sites. *Culex quinquefasciatus* females prefer to oviposit in nutrient-rich water (Frank

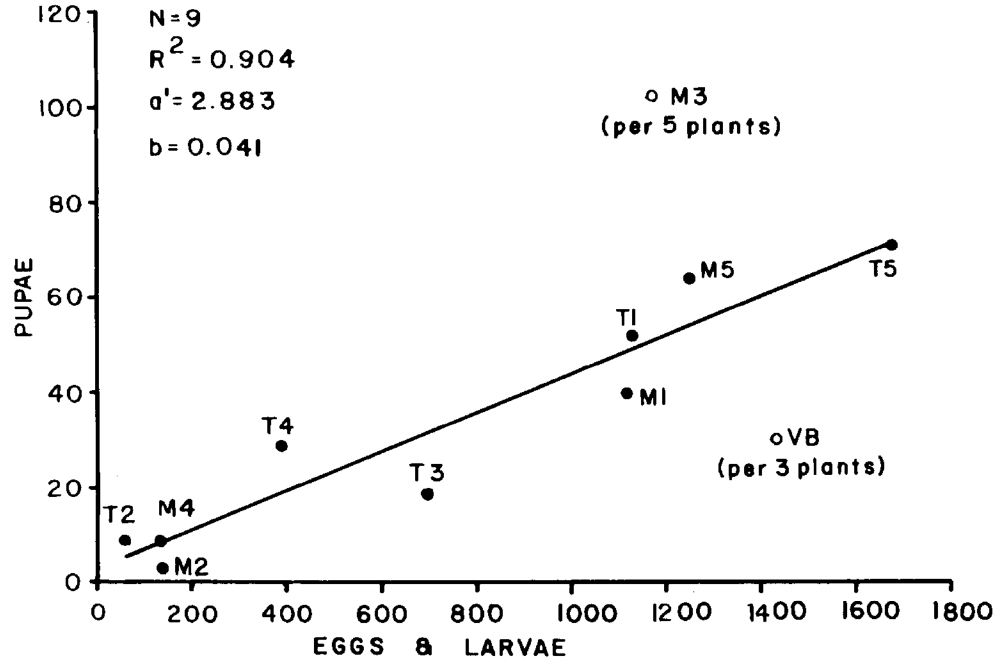

Fig. 3. Scattergram of total no. of *Wyeomyia* pupae collected (y) vs. total no. of eggs and larvae (x) for 5 Tampa sites (T1-T5) and 4 of 5 Miami sites (M1-M2, M4-M5), which were the sites with <1700 eggs and larvae per 9 plants per 12 monthly visits. Data for one Miami site (M3) and a Vero Beach site (VB), which had many more eggs and larvae, are excluded from the fitted linear regression.

& Lynn 1982), which was found in the reservoirs of *B. pyramidalis* only when these contained lawn grass clippings.

Numbers of individuals

Total numbers of *Wyeomyla* eggs + larvae at the Tampa sites and at 4 of the 5 Miami sites were clustered in the range 60-1677. The number of pupae was related to number of eggs + larvae by a linear regression which explained most of the variance (R^2 = 0.904) (Fig. 3). It thus appears that, over this range of densities of eggs + larvae, the number of pupae found depends simply upon the standing crop of eggs + larvae.

The number of eggs + larvae (the standing crop) at the remaining Miami site totalled 2,112, which was well outside the above-mentioned range. When data for this site (M3) were adjusted downward (calculated as no. per 5 plants instead of per 9 plants) to fit the data point onto the graph (Fig. 3), the data point was found to be a significant outlier and was thus excluded from the regression (test for outliers, t = 5.6295, *P* <0.01). Therefore the number of pupae at this site (M3) was significantly greater than predicted. In contrast, although the standing crop of eggs + larvae at Vero Beach (21,441 per 45 plants) was far beyond the range of the calculated regression, when this data point was recalculated as no. per 3 plants, the recalculated number of pupae was smaller than expected (Fig. 3).

The regression shows a linear relationship of number of pupae to standing crop of eggs + larvae within the range 60-1677 of the latter. Above 1677, the direction of the line cannot now be determined, but there are reasons to suspect that it becomes asymp-

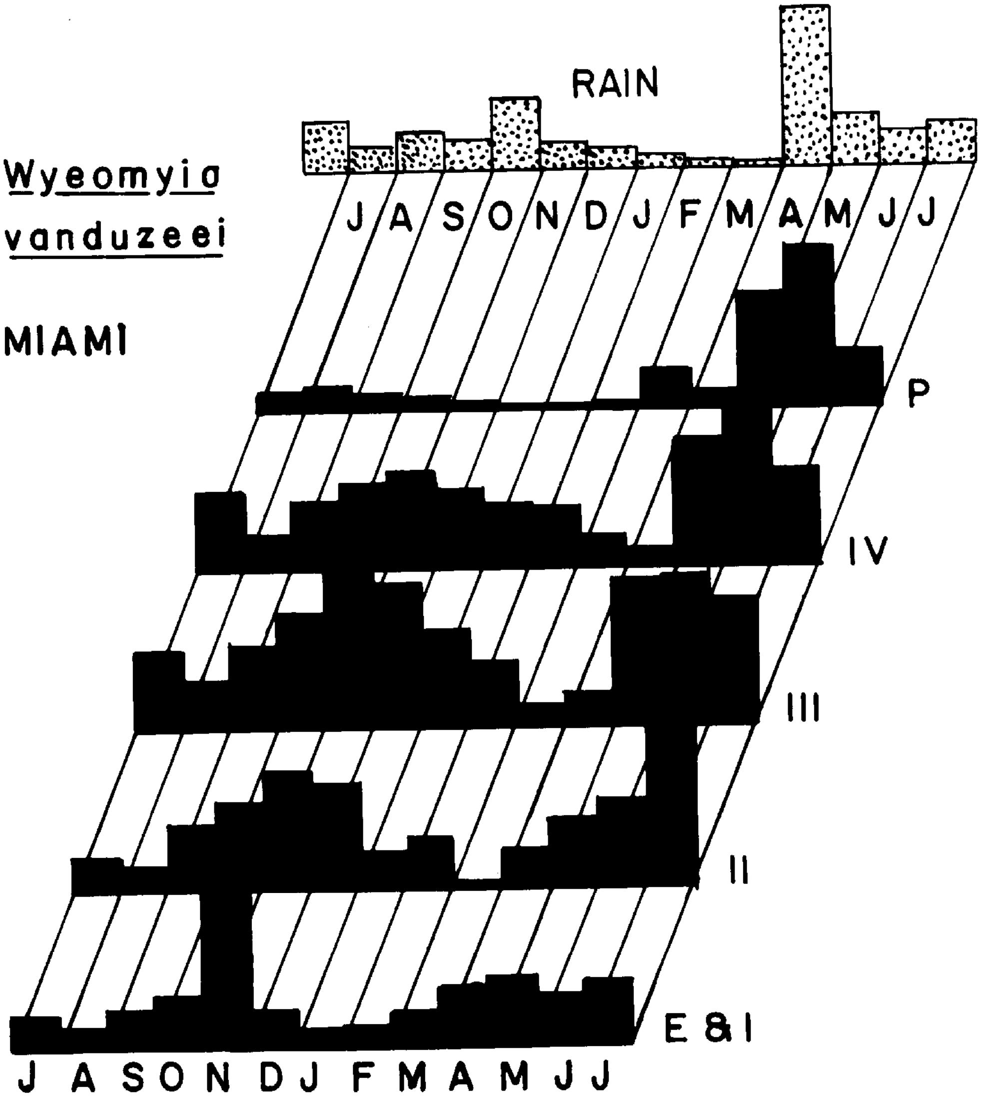

Fig. 4. Histogram showing proportions of the immature stages of *Wyeomyia vanduzeei* collected in Miami at each visit in relation to total monthly rainfall at Miami airport. Height of bars showing number of specimens is normalized.

totic. Pupal production in the laboratory is limited by availability of food to competing larvae (Frank et al. 1985). The Vero Beach site, from which data are the most reliable because of the large number of plants sampled, maintained a very large standing crop of eggs and larvae; perhaps the production of pupae at this site was limited by food, and a smaller proportion of larvae was able to pupate than at the other sites.

It is not clear why there was an large proportion of pupae at one of the Miami sites (M3) as well as a large standing crop of eggs and larvae there. Following the same rationale as with the Vero Beach site, there must have been an unusually abundant food supply. However, because this was one of the least shaded habitats, there was little likelihood of rich food input from tree canopies.

Relationship of pupal production to rainfall

Using all the available data, histograms were drawn for each city and *Wyeomyia* species, showing numbers of aquatic stages collected monthly, and total monthly rainfall (as measured at the closest weather station and published in Climatological Data). The histograms (e.g., Fig. 4) showed the presence of all aquatic stages throughout the year, yet considerable variation in numbers from month to month. They also showed a similarity of pattern of numbers of pupae to rainfall pattern. An analytical program was written in Fortran to inspect the relationship of pupal numbers to rainfall more closely.

The program correlated (y) numbers of pupae collected on the 12 days of sampling in the year August 1978-July 1979, with (x) time-lagged daily rainfall, in single days and blocks of 2-12 days prior to sampling, for the closest weather station. This produced a triangular matrix of 125 correlation coefficients for *W. mitchellii* at each of the 4 cities, and for *W. vanduzeei* at Miami and Vero Beach (numbers collected at Tampa and Daytona Beach were too small to analyze). Axes of the matrix of coefficients were (x) ending day and (y) starting day (expressed as day number before sampling) defining a time block in which daily rainfall was summed. Along its hypotenuse were arranged coefficients resulting from correlation of single daily rainfall totals with pupal numbers, and it was among these that the highest positive coefficients were found. Coefficients from the hypotenuse of each of the 6 matrices are combined into Table 3.

Daily rainfall 7 days before sampling correlated significantly with pupal numbers of both *Wyomyia* species at the Vero Beach site (Table 3). For Miami, the time interval is indicated as 6 days but, making allowance for at least a 1-day delay between the day of sampling and day of examination, during which larvae were alive and developing, the interval should be adjusted to at least 7 days. For Daytona, the time interval is indicated as 5 days. For Tampa, there seems to be no significant correlation, but this may have been an effect of a torrential rain of 293 mm on 8 May 1979 which was a sampling day.

Why should total daily rainfall 5 to 7 days before the day of sampling be correlated with the number of pupae collected on the day of sampling? The method used does not demonstrate any causal relatlonship. On the other hand, rainfall is associated with food input into reservoirs of bromeliads, and this input could enable a cohort of competing late instar larvae to develop to the pupal stage (Frank et al. 1985). The time interval between food input and appearance of pupae could approximate 7 days. This concept could be used as a hypothesis for experimental testing in the field.

CONCLUSION

Cultivation of *B. pyramidalis* in Florida has extended the habitat available to aquatic stages of *Wyeomyia* mosquitoes which are native to Florida. Unquantified observations suggest that similar habitat is provided by other exotic bromeliads of the genera *Aechmea* and *Neoregelia* especially, but also of several other genera. It is unclear whether this increased habitat under- or overcompensates over large areas of Florida for loss of habitat due to rural destruction of native hardwood trees bearing native, epiphytic bromeliads of the genera *Tillandsia* and *Catopsis*. However, *B. pyramidalis* is cultivated in urban settings where *Wyeomyia* mosquito densities are thus increased. Since the adult female mosquitoes bite humans during daylight hours and, at high densities, cause a pest problem, members of the public should be informed of the mosquito-bromeliad association. Members of the public growing numerous *B. pyramidalis* plants and producing therefrom numerous *Wyeomyia* adults may inadvertently be contravening state and/or local statutes designed to control annoyance caused by mosquitoes. The simplest and most effective mosquito control solution is to limit the number of water-impounding bromeliads grown.

TABLE 3. CORRELATION COEFFICIENTS OF *WYEOMYIA* PUPAL NUMBERS VS. DAILY RAINFALL ON SPECIFIED DAYS BEFORE DAY OF COLLECTION. N = 12 FOR EACH COEFFICIENT. PROBABILITY LEVELS: 0.05 (0.576), 0.01 (0.708), 0.001 (0.823).

	Day number before sampling day										
City/species	2	3	4	5	6	7	8	9	10	11	12
Daytona											
W. mitchellii	–0.190	0.136	0.220	0.586	0.203	0.322	0.087	0.222	0.156	–0.277	–0.216
Tampa											
W. mitchellii	–0.033	0.075	–0.354	–0.291	0.234	0.347	–0.225	0.417	–0.338	0.268	0.022
Vero Beach											
W. mitchellii	–0.125	–0.112	–0.287	–0.024	–0.168	0.669	–0.074	0.015	–0.081	–0.292	–0.517
W. vanduzeei	0.038	0.134	–0.085	–0.037	0.125	0.883	0.001	0.338	–0.035	–0.091	–0.269
Miami											
W. mitchellii	–0.479	–0.120	0.383	–0.001	0.709	0.321	0.108	–0.338	–0.048	–0.363	0.392
W. vanduzeei	–0.206	–0.256	–0.085	–0.141	0.812	0.581	–0.221	–0.227	–0.200	–0.179	0.469

ENDNOTES

In Vero Beach, Audrey Frank worked as a voluntary technician in collecting samples when no technician help was otherwise available; G. D. Dodd (Indian River Mosquito Control District) helped to compile, test, and run a Fortran program for time-lagged correlations; J. R. Rey provided a Spanish abstract, and he and L. P. Lounibos and J. R. Linley (Florida Medical Entomology Laboratory) reviewed manuscript drafts while Bonnie Pattok drew a *B. pyramidalis* plant (Fig. 1b). In Miami, N. DeLeon helped to locate sites with *B. pyramidalis,* and J. H. Heidt (Mosquito Control Division) expedited shipments of samples to Vero Beach. In Daytona, E. J. Shepard (East Volusia Mosquito Control District) helped to identify and count samples. In Tampa, J. D. Gorman and D. Taylor (Hillsborough County Mosquito and Aquatic Weed Control) located sites with *B. pyramidalis where* mosquito samples were taken; there, as at Daytona and Miami, homeowners permitted access to their property. We are grateful to all these people. This is contribution no. XVI in a series on the bionomics of bromeliad-inhabiting mosquitoes. University of Florida, Institute of Food and Agricultural Sciences, journal series no. 8337.

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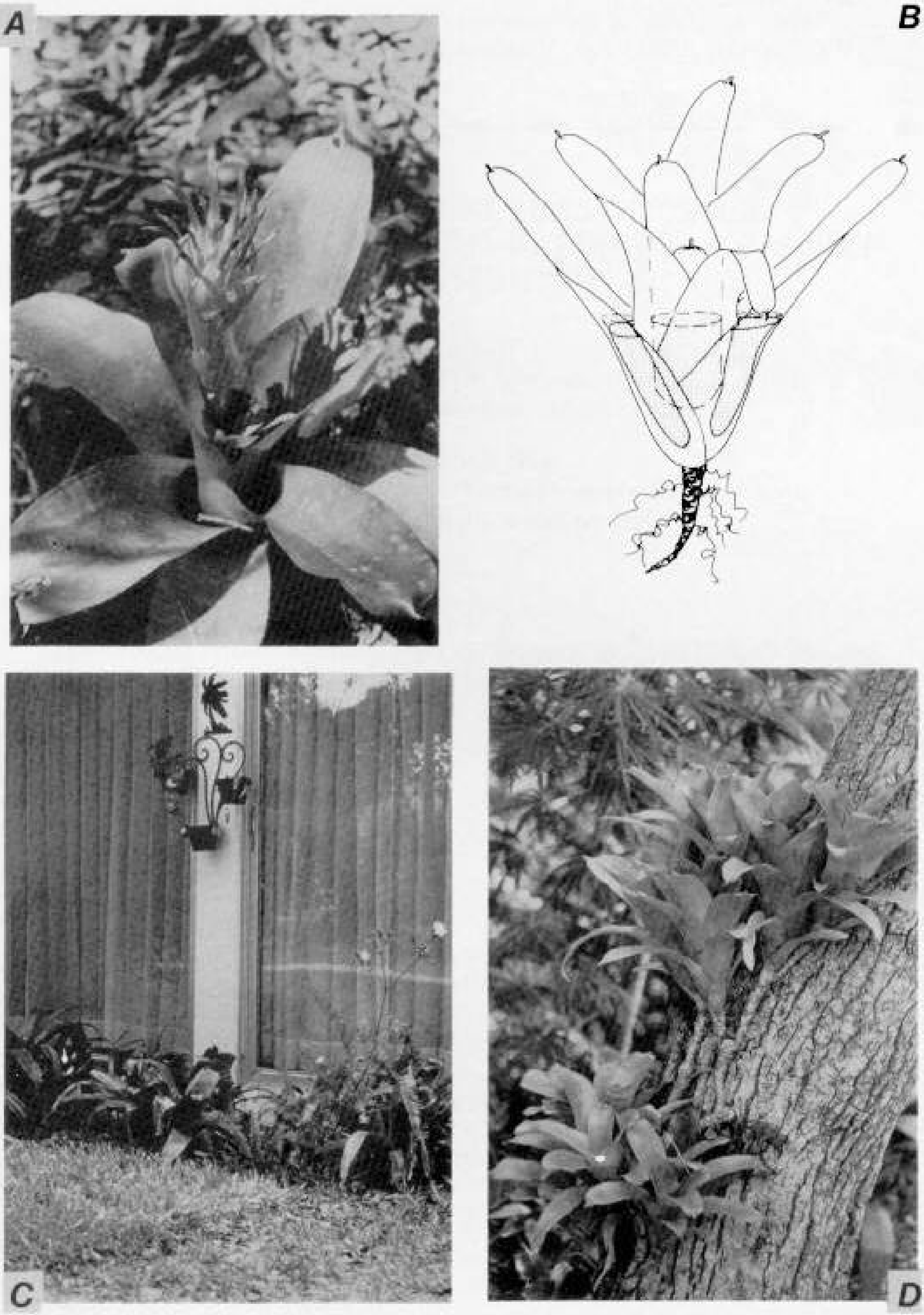
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