EVALUATING ULTRA-LOW VOLUME GROUND APPLICATIONS OF MALATHION AGAINST *AEDES AEGYPTI* USING LANDING COUNTS IN PUERTO RICO, 1980–84

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ABSTRACT. Landing counts made in a residential area in San Juan, Puerto Rico during the 5-year period, 1980–84 indicated that malathion ULV applied by LECO Fog Generators was not effective against *Aedes aegypti*. Of 25 applications only 6 resulted in a decrease on the first day after treatment and only one remained much less than the pretreatment count on the second and third days after treatment. Daily, the number of mosquitoes landing for a 5-minute period at each of 2 stations was recorded. An ideal year created by averaging the monthly data suggested that the seasonal variation of mosquitoes was inverse to that of reported dengue incidence.

INTRODUCTION

Previous studies indicated that ultra-low volume (ULV) ground applications of malathion in 1978 and 1979 were not effective against *Aedes aegypti* (Linn.) in Puerto Rico (Fox 1980a, 1980b); nevertheless, federal and local public health officials chose to continue the program in subsequent years. The purpose of the present research was to find out: (a) the effect of ground ULV sprayings during the 5-year period, 1980–84 on a segment of the natural adult population of *Ae. aegypti* biting man in the city of San Juan, and (b) whether or not variation in the landing counts could be associated with the seasonal pattern of dengue incidence as reported by the Puerto Rico Department of Health each month.

MATERIALS AND METHODS

The study period was from January 1, 1980 through December 31, 1984, and the study area was in a residential part of the city of San Juan known as Santurce in the section called Ocean Park. During the study period in the evenings between 1800 and 2015 hr P.R. Department of Health personnel treated the study area 27 times using LECO Model HD Fog Generators dispensing malathion 96% at 7.57 liter/hr. In the study area there were 2 specific landing count sites located in the back patio of a house on a street where the LECO fog generators passed about 17 m away. Ocean Park is a well maintained neighborhood of mostly one-family residents in one-, rarely 2-story cement houses. Typically, the houses occupy small lots, ca. 260 m^2, and are separated by driveways and open spaces, ca. 5 m wide on each side; there are 7 or less houses of similar design per block, each with small front and back patios. The patios are well tended with few potential breeding places consisting of potted plants and miscellaneous temporary containers filled by rainfall. The patio used for the landing counts was representative of the neighborhood. It was small, ca. 5 × 8 m; to the back was a high wall and 2-story building, but the LECO fog generator passed in the front where there was only a cement fence ca. 1 m high, and wide open areas between the street and the patio, partly obstructed by vegetation. The landing count sites were at the side of the house and not obstructed by it. Station No. 1 was the same as that used in 1979; Station No. 2 was located about 3 m from Station No. 1, closer to the street where the fog generators passed. Landing counts were made wearing a short-sleeved shirt and shorts, with the arms, legs, and feet bare, standing up, motionless. Every day, weather permitting, one of us (I. F.) counted the mosquitoes landing on him for a period of 5 minutes at each station and although the same ones may have landed more than one time, each landing was counted separately. One specimen was collected at each station and identified each day, when possible. The patio was searched regularly for larvae, and none were permitted to breed there. The daily counts were averaged monthly to find out any relationship to the number of cases of dengue indicated by the Department of Health in their monthly report, *Informe Epidemiologico*. The authorities relied on the reported cases of dengue to implement and evaluate the *Ae. aegypti* control program.

RESULTS

The results of the landing counts made 1 to 3 hours before each treatment (Day 0) and on each of 4 days after each treatment, and the dates are shown in Table 1. Not included in Table 1 are treatments made on April 24 and May 29, 1980 because heavy rains prevented some indispensable landing counts. Of the 25 treatments studied over the 5 years, there were 19 when the landing counts remained the same as the pretreatment counts or increased on the first day after treatment, and only 6 (Numbers 1, 2, 4, 6, 16, and 24) where the posttreatment counts decreased. Of these 6 only one (No. 1) remained much less than the pretreatment

Table 1. Total number of *Aedes aegypti* mosquitoes landing on a man for a period of 5 minutes at 2 stations before treatment (Day 0) and each of 4 days after treatment with ground malathion ULV fogging in San Juan, P.R., 1980–84.

		Days after treatment				
Treatment	Date	0	1	2	3	4
1	June 3, 1980	27	12	19	8	5
2	Aug. 14	3	0	9	4	2
3	Aug. 18	2	4	4	10	4
4	Oct. 23	7	3	24	19	27
5	Oct. 28	15	16	4	9	12
6	Nov. 26	16	10	20	12	36
7	Dec. 4	0	2	ND	0	0
8	Jan. 15, 1981	0	0	4	11	9
9	Feb. 5	9	11	0	6	1
10	Feb. 10	6	10	2	6	0
11	Mar. 2	0	4	4	9	15
12	Aug. 27	2	13	3	2	31
13	Sep. 17	15	15	26	20	35
14	Nov. 9	18	26	28	13	24
15	Nov. 16	22	26	26	19	22
16	Nov. 24	4	2	4	12	5
17	Nov. 30	1	2	6	7	18
18	Jan. 18, 1982	14	28	25	35	38
19	Jan. 21	35	38	40	24	30
20	Feb. 10	11	21	5	24	3
21	Feb. 16	20	22	10	23	ND
22	Aug. 16	4	5	10	7	0
23	Feb. 17, 1983	5	5	6	7	0
24	May 8, 1984	27	17	ND	26	14
25	June 20	37	41	61	63	73

ND = Not done.

count on the second and third days after treatment. The average of all the Day 1 posttreatment counts, 13.3, was greater than the average of all the pretreatment counts, 12.0. While making the landing counts shown in Table 1, 81 specimens were collected, all *Ae. aegypti*, of which 73 were females and 8 were males.

Table 2 records the average number of *Ae. aegypti* mosquitoes landing per day per month, the standard deviation, and the range at each of the 2 stations during the 5-year period, 1980–84. The landing counts indicated a definite seasonal variation in abundance favoring the months of May, June, July, and sometimes August, when the averages per day were highest, while the low averages occurred usually in the months of October, November and December. Table 3 shows for each year the total number of days the landing counts were made, the total number of mosquitoes landing, and the average per day per year at each station. To find out if the pattern of dengue incidence could be related to the seasonal variation in abundance of mosquitoes, an ideal year was created by averaging the data in Table 2 and the number of cases of dengue reported each month. The results are shown in Fig. 1.

The total number of mosquitoes collected while landing during the study period was 1,296 of which 1,286 were *Ae. aegypti* (175 males, 1,111 females) and 10 were females of several other species; therefore 99% of the mosquitoes collected were *Ae. aegypti*. The total number of mosquitoes collected each year were as follows: 1980, 180; 1981, 226; 1982, 267; 1983, 271; and 1984, 352.

DISCUSSION

Only hungry female *Ae. aegypti* are directly involved in the transmission of dengue, therefore the present study has been limited to assessing that segment of the adult population in the act of attacking humans. The most reliable method for monitoring blood-seeking mosquitoes is routine collections of mosquitoes attracted to human bait (Service 1977). Other methods do not measure the mosquitoes in the proper stage of development if the method depends on egg or larval collections, or proper phase of activity if the method depends on trapping adults in general. From ovitrap data it can be inferred that human blood-seeking females once were abundant, but the opposite is not certain; that is, the absence of eggs on the ovitrap paddles does not prove the absence of human blood-seeking females. Landing counts in Puerto Rico on occasion have proved the presence of numerous mosquitoes attacking man even though ovitrap paddles were negative (I. Fox, unpublished data). Because *Ae. aegypti* may not oviposit in sites contaminated by insecticides (Moore 1977), it is unwise to rely on ovitraps to indicate the percentage killed by an insecticide.

During the 5-year study period involving a total of 3,395 sessions in 1,696 days, not a single week passed when no mosquitoes landed, and there were only 143 days when the counts were zero at both stations. Therefore, the present study shows that in an area of high *Ae. aegypti* density, routine landing counts of only 5-minute periods at each of 2 stations, and collecting one mosquito at each station, were sufficient to indicate the presence of *Ae. aegypti* 90% of the time. The data in Table 2 provide base lines for comparison in the future, should other insecticides or methods of control be tried in the study area.

The literature indicates that malathion ULV is effective against *Ae. aegypti* in certain situations depending upon dosage, technique of application and frequency of treatment (Pant et al. 1971). However, at the dosage permitted in the U. S. A., Focks et al. (1987) reduced females by only 30%, as estimated from results of ovitraps and adult traps. One ULV treatment using U.S.A. label dosages should kill at least 70% of

Table 2. Average number (±SD) of *Aedes aegypti* mosquitoes landing on a man for a period of 5 minutes at 2 stations separated by about 3 m in San Juan, P.R., 1980–84.

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
1980						*Station No. 1*						
Mean ± SD	2.7 ± 3.7	0.5 ± 1.2	0.9 ± 1.6	2.3 ± 4.4	12.5 ± 11.8	4.6 ± 5.9	4.2 ± 3.8	1.6 ± 2.2	1.8 ± 2.6	3.9 ± 4.7	3.1 ± 3.0	2.2 ± 3.2
Range	0–13	0–4	0–7	0–21	0–48	0–21	0–15	0–8	0–10	0–20	0–13	0–13
						Station No. 2						
Mean ± SD	8.3 ± 7.0	4.2 ± 5.4	2.5 ± 3.2	5.6 ± 6.7	23.8 ± 16.8	10.7 ± 6.6	8.9 ± 6.1	4.5 ± 4.7	4.0 ± 4.9	7.4 ± 6.8	5.1 ± 5.1	3.3 ± 3.6
Range	0–32	0–14	0–10	0–27	2–70	0–26	1–26	0–20	0–23	0–31	0–23	0–13
1981						*Station No. 1*						
Mean ± SD	1.6 ± 2.4	2.0 ± 2.4	2.5 ± 2.8	3.2 ± 3.8	4.0 ± 4.1	5.5 ± 4.6	6.9 ± 4.7	8.2 ± 6.5	6.9 ± 4.2	2.9 ± 3.8	5.4 ± 5.2	5.4 ± 6.2
Range	0–10	0–11	0–9	0–12	0–14	0–20	0–17	0–25	0–17	0–13	0–24	0–21
						Station No. 2						
Mean ± SD	3.8 ± 3.8	4.8 ± 4.0	4.9 ± 4.3	4.4 ± 3.4	7.4 ± 4.9	6.7 ± 3.7	11.0 ± 6.2	10.7 ± 6.5	10.6 ± 6.5	5.7 ± 5.4	9.2 ± 6.6	9.2 ± 10.3
Range	0–12	0–14	0–13	0–12	0–18	0–15	0–22	0–26	0–24	0–17	0–29	0–41
1982						*Station No. 1*						
Mean ± SD	9.1 ± 6.3	4.0 ± 4.3	5.2 ± 5.1	3.4 ± 3.1	7.0 ± 7.3	16.1 ± 12.4	7.4 ± 5.4	4.3 ± 3.3	4.8 ± 4.5	2.8 ± 2.3	5.2 ± 3.7	6.5 ± 5.4
Range	0–30	0–15	0–23	0–10	0–32	0–46	0–25	0–13	0–16	0–9	0–15	0–16
						Station No. 2						
Mean ± SD	13.4 ± 7.3	9.0 ± 5.4	10.5 ± 7.4	3.8 ± 2.9	9.0 ± 7.2	18.3 ± 13.6	11.8 ± 5.8	7.2 ± 5.7	5.7 ± 5.6	3.1 ± 3.2	7.0 ± 4.7	7.3 ± 5.5
Range	0–34	0–23	0–26	0–11	0–28	0–50	2–25	0–21	0–20	0–12	0–16	0–18
1983						*Station No. 1*						
Mean ± SD	3.0 ± 3.1	2.5 ± 2.3	4.2 ± 5.5	9.3 ± 13.8	23.4 ± 13.3	27.0 ± 6.4	11.4 ± 8.3	4.1 ± 3.1	1.6 ± 2.3	2.8 ± 3.2	1.6 ± 2.1	3.2 ± 3.5
Range	0–12	0–10	0–25	0–45	3–50	13–40	1–41	0–11	0–9	0–13	0–6	0–14
						Station No. 2						
Mean ± SD	6.0 ± 6.0	4.3 ± 3.1	6.0 ± 5.8	11.6 ± 14.9	26.7 ± 14.2	29.7 ± 6.4	14.1 ± 10.2	5.6 ± 3.3	2.8 ± 2.9	3.1 ± 3.4	2.0 ± 2.5	4.5 ± 4.0
Range	0–20	0–11	0–27	0–48	3–60	14–40	2–56	0–11	0–14	0–15	0–9	0–14
1984						*Station No. 1*						
Mean ± SD	2.8 ± 3.3	2.2 ± 2.8	4.9 ± 6.6	8.7 ± 5.7	7.4 ± 4.7	18.6 ± 10.3	27.9 ± 14.7	29.5 ± 27.0	24.9 ± 21.2	4.3 ± 4.1	1.3 ± 1.4	1.3 ± 2.9
Range	0–12	0–13	0–37	0–21	0–21	1–36	6–54	0–56	1–70	0–19	0–4	0–14
						Station No. 2						
Mean ± SD	3.6 ± 3.3	3.0 ± 3.5	5.7 ± 7.2	12.0 ± 6.0	10.4 ± 6.0	25.7 ± 11.7	32.3 ± 16.6	29.0 ± 27.1	19.9 ± 18.5	2.9 ± 2.4	1.8 ± 3.1	1.3 ± 2.1
Range	0–12	0–10	0–40	3–23	2–29	5–56	5–59	0–80	0–50	0–17	0–14	0–9

Table 3. Number of *Aedes aegypti* mosquitoes landing on a man during 5 minutes at each of 2 stations, the number of days the counts were made, the years, and the average per day per year.

	Station No. 1			Station No. 2		
Year	Days	Mosquitoes	Avg./day	Days	Mosquitoes	Avg./day
1980	355	1,195	3.5	355	2,618	7.4
1981	342	1,553	4.5	341	2,418	7.3
1982	337	2,139	6.4	336	2,967	8.9
1983	341	2,672	7.8	340	3,305	9.7
1984	324	3,903	12.0	324	4,351	13.4
Total	1,699	11,462	6.7	1,696	15,721	9.3

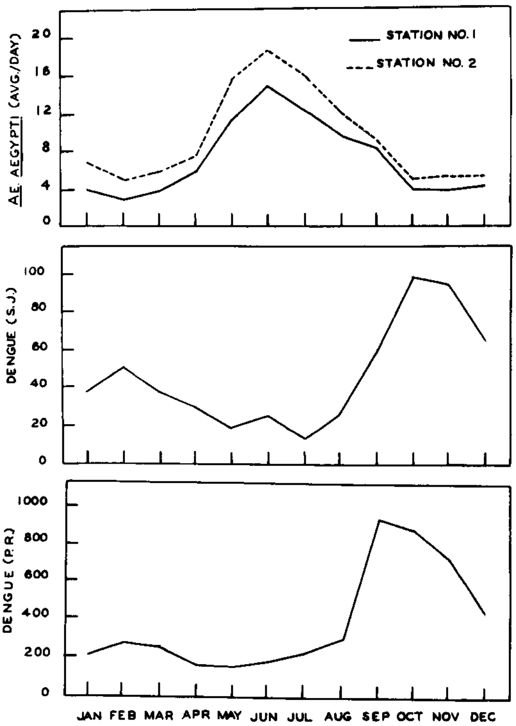

Fig. 1. An ideal year created by averaging the data of each month in Table 2 and the number of cases of dengue reported each month for the years 1980 through 1984. The average number of *Aedes aegypti* mosquitoes per day per month landing on a man during 5 minutes each at 2 stations (top), the number of cases of dengue reported each month from San Juan (S. J.) and vicinity (middle), and the number of cases of dengue reported each month from Puerto Rico (P. R.) as a whole (bottom).

the mosquitoes in a range of 91 m; if such a kill is not obtained another insecticide should be used (Anonymous 1977). Of the 25 applications studied here, only one, No. 2, August 14, 1980 (Table 1) indicated a kill of more than 70%, but by Day 2 the mosquitoes were more abundant than before the treatment.

Mosquitoes less than 24 hours old probably do not engage in flight to seek blood (Pant and Yasuno 1973). Therefore, it is likely that post-treatment counts made less than one day after pretreatment counts represent the same population of adult mosquitoes without additions due to recent eclosions. The ULV malathion treatments discussed here did not reduce the *Ae. aegypti* adult population in the area sprayed, for the number landing after the spraying was most of the time not less than the number landing before the sprays. It is possible that the sprays were in fact harmful for more times than not (14 of 25), the number which landed after the treatments increased, suggesting that the spray had the effect of stimulating the mosquitoes to greater activity. However, this could not be confirmed by statistical analysis.

The data in Table 1 were analyzed statistically using the SPSS-X version 2.1 computer package on the IBM 4361 computer. In the 22 complete groups, the means of mosquito landing counts showed a slightly increasing trend, Day 0, 11.50; Day 1, 13.27; Day 2, 15.00; Day 3, 14.86; and Day 4, 17.73. An overall analysis of variance was performed using the ONEWAY procedure with *a posteriori* contrast tests, LSD, DUNCAN, SNK, TUKEYB, TUKEY, LSDMOD, and SHEFFE. The overall F-test was not significant ($P = 0.6737$), also none of the contrast tests were significant at the 0.05 level. The NPAR TESTS FRIEDMAN and KENDALL were used to see if there was any concordance in the rank order of the number of mosquitoes in each of the days of each spraying period. The Friedman two-way ANOVA, and the Kendall coefficient of concordance were not statistically significant. This indicates that there were no consistent changes in the mosquito landing counts over the days immediately following the spraying. Using the data from the 22 complete records, we summed the total number of mosquitoes for each of the days. The Chi-square test gave $P = 0.000$, indicating significant differences among the totals. However, in very large samples even small deviations generate a significant Chi-square (Nie et al. 1970, p. 224). This may be due to inhomogeneity of the data rather than any real relationship. To examine the distribution of the data, we used the NPAR TESTS (K-S) which evaluate the probability that the distribution is uniform, normal, or poisson. All these tests were significant, indicating that the distribution does not fit any theoretical pattern. The statistical analysis indicates that there was no important effect of the spraying on the number of mosquitoes observed.

Moore et al. (1978) monitored the seasonal variation of larvae in breeding sites around houses in 5 communities in Puerto Rico but not in San Juan. After finding that larval densities varied with rainfall, they concluded that there was a close correlation between larval abundance and dengue incidence. While the assumption that numbers of adults will increase when the larvae increase due to rainfall may be valid, the theory that there is a positive relationship between the abundance of larvae or adults and dengue incidence is challengeable. A previous study (Fox 1980a) indicated that in San Juan larvae were usually abundant, but severe outbreaks of dengue were rare. The present study yielded similar data concerning adults. In San Juan and vicinity the number of cases reported each year during the study period were: 1980, 217; 1981, 1357; 1982, 860; 1983, 103; and 1984, 76. Table 3 shows that the year with most mosquitoes, 1984, was the year with the least number of dengue cases reported, while 1981 with most cases of dengue had fewer mosquitoes than the other years.

The ideal year shown in Fig. 1 suggests that both the blood-seeking mosquitoes and the reported cases of dengue had a definite seasonality, but that there was an inverse relationship between them; the peak months of the landing counts often being the troughs of dengue incidence. May, June, July and August were the months with the highest landing counts, while September, October, November and December were the months with the greatest number of reported cases. The lag of several months shown between the beginning of the period of high numbers of reported cases and the period of high numbers of blood-seeking mosquitoes suggests that using the incidence of dengue to determine when and where to spray was of doubtful benefit not only because the spray was ineffective, but also because the control forces were mustered when the mosquitoes were already on the wane after enjoying a lengthy period of abundance.

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