FIELD STUDIES OF GARDONA AGAINST *AEDES AEGYPTI* IN PUERTO RICO

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In the chemical control operations of the Aedes aegypti Eradication Program in Puerto Rico during 1965 to 1968, the principal insecticide used for premises treatment was malathion, since the species was resistant to both DDT and dieldrin (Flynn *et al.*, 1964). The chemical was dispensed by hand and/or power equipment as a 2.5 percent emulsion which was applied to all containers holding nonpotable water, such as bottles, tires and tin cans; treatment was made on a block by block basis. Since laboratory and simulated field tests had indicated other organophosphorus compounds to be superior to malathion, such chemicals were field-tested on a small scale in Puerto Rico and at Perrine, Florida. In Florida (Eliason and von Windeguth, unpublished manuscript) three compounds, Abate,[6] Gardona and bromophos, were shown to be superior to malathion as a larvicide against *Aedes aegypti* breeding in small containers. Limited tests with bromophos and malathion in two municipios in Puerto Rico also suggested that malathion was less effective. In view of these promising results, emphasis was placed on conducting a large-scale evaluation of one of the three above mentioned compounds in two municipios in Puerto Rico. Since the biological efficacy of the three compounds against *Ae. aegypti* was similar, selection of the test insecticide was based on market availability, registration, mammalian hazard, and cost of each compound. On the basis of these criteria, Gardona was chosen as the test insecticide for the 1968 studies reported in this paper.

MATERIALS AND METHODS

(a) STUDY AREAS. Three municipios, Corozal, Naranjito and Morovis, 15 to 20 miles west of San Juan, Puerto Rico, none of which had a history of previous treatment by the Aedes aegypti Eradication Program, were selected as experimental areas (Figure 1). Corozal consisted of one urban community (1,340 premises) and 12 rural barrios (625 premises). Naranjito had a single urban community (800 premises) and seven rural barrios (776 premises). Morovis, where only the urban area of 750 premises was involved, served as an untreated town in the first round of spraying and as a treated community in the second spray cycle.

(b) CONTROL PROCEDURES. All urban and rural premises in Corozal and Naranjito were treated with Gardona suspension. In the first spray cycle[7] a 1.25 percent strength was used in the former, 2.5 percent in the latter. In the second spray

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[6] Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health, Education, and Welfare.

[7] The first spray cycles for urban Corozal and Naranjito were April 1 to May 13 and April 15 to May 9, respectively. The rural evaluative areas in Corozal and Naranjito were treated May 9 to 13 and May 10 to 28, respectively.

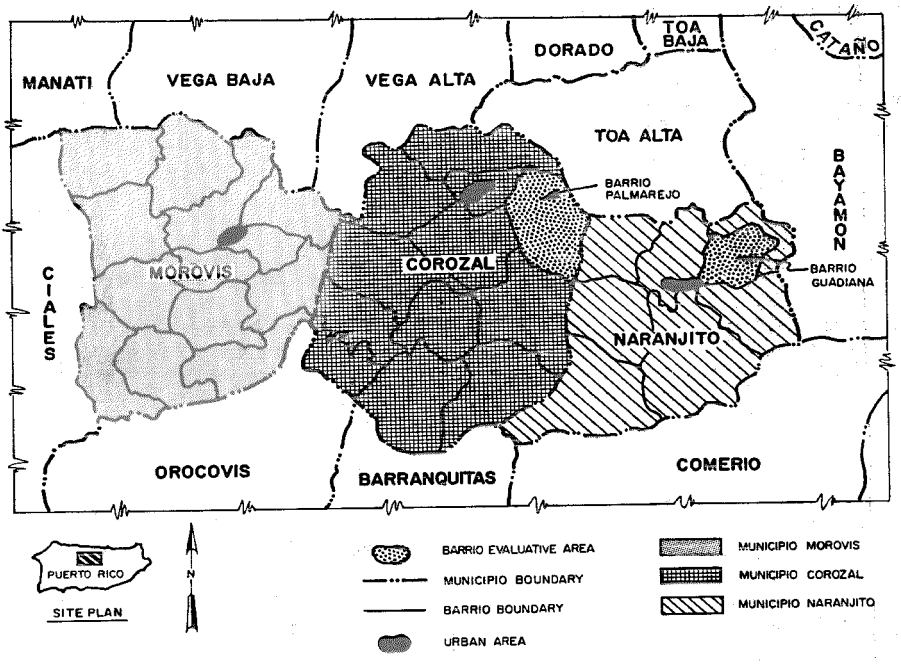

FIG. 1.—Map of areas.

cycle [8] both municipios were sprayed with 2.5 percent suspension. The required "charge" of Gardona was carried by each sprayman in individual plastic bags so that one charge in a hand sprayer can plus 2 gallons of water provided the correct concentration. Most of the premises were treated by this method, but power equipment was used for spraying trash dumps and open lots. Potable water and animal watering pans were dosed with 1 percent Abate sand granules, and dichlorvos resin strips were installed in the few cisterns involved.

Application procedures followed those normally employed in control operations in Puerto Rico except that no insecticides were used in the interior of houses and one individual of each two-man spray crew marked the containers with yellow paint before spraying them. This marking was necessary to enable the evaluation inspector to be certain that the container inspected had been treated, a procedure unnecessary in regular spray operations. In addition, spraymen frequently agitated their spray cans to prevent settling of the suspension, a procedure not followed with the emulsion formulation used on the operational program. Because of the large area involved in the rural portion of each municipio, one barrio in each was selected to represent the remaining rural zone. Barrio Palmarejo (87 premises) in Corozal and Barrio Guadiana (93 premises) in Naranjito were chosen. The former represented 14 percent of the total rural premises, the latter, 12 percent. These barrios

[8] The second spray cycle for Corozal was August 28–September 21 and for Naranjito, September 19–28.

were treated the same week, and all inspections in the rural areas were conducted therein.

(c) ENTOMOLOGICAL EVALUATION. Survey teams inspected containers on the premises, open lots and dumps in the urban areas and recorded whether they were positive or negative for mosquito breeding. Except for tremendous numbers of containers at the dump sites, all containers were checked. Records were maintained to show whether the container found was marked (*i.e.*, painted yellow) or unmarked, wet or dry, and positive or negative for *Aedes* larvae (*aegypti* or *mediovittatus*). All containers reported as positive for *Ae. aegypti* were also inspected and confirmed by the supervisor.

Ovitraps (Fay and Eliason, 1966; Jakob and Bevier, 1969) also were used in the same areas where larval surveys were conducted. Distribution was on a 350-foot grid pattern in the urban areas and the same design was followed in rural areas where feasible. With either method pretreatment assessments were made. Posttreatment larval surveys were scheduled at monthly intervals, but in practice the periods occurred from 4 to 5 weeks apart. Ovitraps were inspected weekly.

(d) EVALUATION OF CHOLINESTERASE LEVELS. To detect possible effects of the Gardona application on the spraymen, blood samples were taken from an initial group of 30 persons. The number unavoidably decreased, and the ultimate group that was consistently checked over the first spray cycle consisted of 16 persons. The local health department collected blood samples by venal puncture from the spraymen at intervals ranging from 1 to 3 weeks. The blood samples taken in vacutainers containing sodium heparin were centrifuged, separated and refrigerated, and the cell fractions were shipped to Perrine, Florida, where cholinesterase determinations were performed by the Perrine Primate Laboratory. The pH stat method, as described by Nabb and Whitfield (1967), was used to determine the values.

RESULTS

FIRST SPRAY CYCLE

Municipio Corozal: In urban Corozal pretreatment surveys were conducted March 4–8 and March 18–22 with larval indices as shown inTable 1. In the 10,091 containers surveyed, the average percentage of containers positive for larvae of *Ae. aegypti* was 7.4. A total of 1,266 premises were sprayed with 1.25 percent Gardona suspension by using hand units and 215 by power equipment. Approximately 49,000 containers were sprayed. Two weeks previously, of 4,267 containers with water, 6.1 percent were positive for *Ae. aegypti* larvae. Five weeks after treatment the index for the treated ones was 0.08 percent; at 10 weeks it rose to 1.1. On the first posttreatment inspection 24,482 containers were dry, on the second, 19,112. The data indicate that 2,505 and 2,215 wet unmarked or untreated containers were detected on the first and second posttreatment surveys, respectively, of which 1.9 and 4.8 percent were positive for *Ae. aegypti* larvae. In comparison with the treated cans, the in-

TABLE 1.—Percent of premises and wet containers positive for *Aedes aegypti* larvae in urban Corozal, first spray cycle, of 1.25 percent Gardona suspension.

Inspection (weeks)	Premises		Treated Containers		Untreated Containers		All Containers	
	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.
−4	1258	20.6	....	....	6824	7.2	6824	7.2
−2	1283	11.6	....	....	4267	6.1	4267	6.1
Treatment [1]								
+5	1283	2.9	3704	0.08	2505	1.9	6209	0.8
+10	1283	8.3	2490	1.1	2215	4.8	4705	2.8

[1] April 1–11, 1968.

festation rates for the untreated wet containers were 23.7 and 4.3 times higher. In untreated Morovis (Table 2) the larval positive indices for the containers marked during inspection I were 19.1 and 19.7 percent. On inspections II and III unmarked containers in Morovis showed an average 16.0 and 17.5 percent to be positive for larvae.

In the rural area of Corozal, Barrio Palmarejo, 87 premises were hand sprayed; 17 received the treatment by power equipment. In this area the pretreatment index for larval positive containers was 1.9 percent (Table 3). At 5, 9, 13, 17 and 21 weeks after treatment the indices for wet treated containers were 0, 0, 0, 0.8 and 0 percent, respectively. For the same successive weeks the indices for wet untreated containers were 0, 1.1, 2.7, 2.2 and 2.1 percent.

The data from the 75 ovitraps dispersed in urban Corozal revealed that 10.6 to 17.8 percent were positive for eggs of *Ae. aegypti* during weeks 10 to 13. During the spraying period of week 14 through week 19 the weekly indices for positive ovitraps were 13.2, 15.8, 13.2 8.0, 6.6 and 9.2 percent. On week 20 the index dropped to 1.3 percent, but the following week it rose to 17.3 percent and remained at levels between 11.3 to 21.3 percent for the next 3 weeks. For the weeks 17 to 20 the percentage of positive traps remained below 10.

In Barrio Palmarejo pretreatment ovitrap indices for a 5-week period indicated that eggs were present in 3.1 to 18.8 percent of the 32 traps used. After treatment the ovitrap indices fluctuated from 0 to 9.7 percent, and none of the traps were positive on 4 of the 9 posttreatment weeks.

TABLE 2.—Percent of premises and wet containers positive for *Aedes aegypti* larvae in untreated Morovis, first spray cycle.

Inspection No.	Premises		Marked Containers		Unmarked Containers		All Containers	
	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.
I [1]	712	25.1	....	....	2925	15.9	2925	15.9
II [2]	686	11.7	1825	19.1	868	16.0	2693	18.5
III [3]	710	22.0	1173	19.7	926	17.5	2099	18.7

[1] Inspection and marking of containers April 22–27, 1968.
[2] Inspection 5 weeks after [1].
[3] Inspection 10 weeks after [1].

TABLE 3.—Percent of premises and wet containers positive for *Aedes aegypti* larvae in Barrio Palmarejo, Municipio Corozal, first spray cycle, of 1.25 percent Gardona suspension.

Inspection (weeks)	Premises		Treated Containers		Untreated Containers		All Containers	
	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.
<1	87	10.3	..	..	776	1.9	776	1.9
Treatment [1]								
+5	86	0	77	0	63	0	140	0
+9	84	4.8	164	0	548	1.1	712	0.8
+13	83	6.0	105	0	182	2.7	287	1.7
+17	84	6.0	132	0.8	268	2.2	400	1.8
+21	84	7.1	130	0	329	2.1	459	1.5

[1] April 29–May 3, 1968.

In urban Corozal the ovitraps also were positive for eggs of *Ae. mediovittatus,* and indices for this species followed the same general trends as for *Ae. aegypti* except that they were slightly lower. In Barrio Palmarejo, however, the oviposition findings suggested that *Ae. mediovittatus* was much more prevalent, since indices of 40 percent or greater were recorded for 11 of the 15 survey weeks. The insecticide treatment thus appeared to have less effect on this species than on *Ae. aegypti.*

Municipio Naranjito: In urban Naranjito pretreatment surveys were made March 11 to 13 and March 25–26 (Table 4). The combined index for these two surveys in wet containers showed that 1.3 percent of the containers were positive for larvae of *Ae. aegypti.* Approximately 749 premises were then treated with hand sprayers; 78 with power units. The number of containers sprayed totalled 51,575. At the 5-week posttreatment inspection 1,157 containers were wet, but all were negative for *Ae. aegypti* larvae. The inspections at 9 and 13 weeks after spraying showed indices in the wet treated containers of 0 and 0.3 percent. Untreated wet containers showed indices of 0.1, 0.4 and 0.7 at the 5-, 9- and 13-week inspections, respectively. Indices for untreated Morovis are given in Table 2.

In Barrio Guadiana, the rural evaluative portion of this municipio, 93 premises were hand sprayed; 52 were treated with power equipment. The pretreatment index for the 262 wet containers was 6.8 percent (Table 5). Posttreatment inspections at 5, 9, 13, 17 and 21 weeks were negative except for two positive containers detected at week 21. Untreated wet containers in this area had indices ranging from 0.8 to 3.8 over the same time span.

Ovitrap indices for *Ae. aegypti* in urban Naranjito did not exceed 4.5 percent for any of the pre- and posttreatment weeks from 10 to 28, and no marked change occurred during the treatment period. In contrast, indices of 14 to 41 percent were recorded for *Ae. mediovittatus,* but the treatment exerted no apparent detrimental effect on oviposition. In Barrio Guadiana, the ovitrap index for *Ae. aegypti* eggs ranged from 0 to 6.4 percent, but the treatment apparently had little influence on oviposition. *Ae. mediovittatus* eggs were recovered from 17.0 to 45 percent of the 47 ovitraps during the 12-week observation period.

TABLE 4.—Percent of premises and wet containers positive for *Aedes aegypti* larvae in urban Naranjito, first spray cycle, with 2.5 percent Gardona suspension.

Inspection (weeks)	Premises		Treated Containers		Untreated Containers		All Containers	
	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.
−5	778	3.3	..	..	2085	1.5	2085	1.5
−3	765	2.5	..	..	1803	1.1	1803	1.1
Treatment [1]								
+5	770	0.1	1157	0	794	0.1	1951	0.05
+9	768	0.4	607	0	740	0.4	1347	0.2
+13	755	1.0	311	0.3	1171	0.7	1482	0.6

[1] April 15–19, 1968.

One of the obvious factors apparent in both municipios was the rapid turnover in containers. Data are given in Table 6 which demonstrate this point. In urban Corozal the inspection 5 weeks after treatment revealed that of 48,771 containers inspected 42 percent represented unmarked containers that were not present when the area was sprayed. On the survey at 10 weeks, 52 percent of the 45,151 containers were new. In Naranjito the same progressive increase in new or unmarked containers occurred, as was true for the rural areas of each municipio and for untreated Morovis.

During the first spray cycle (April 1 through May 28) a total of 8,153 gallons

TABLE 5.—Percent of premises and wet containers positive for *Aedes aegypti* larvae in Barrio Guadiana, Municipio Naranjito, first spray cycle, with 2.5 percent Gardona suspension.

Inspection (weeks)	Premises		Treated Containers		Untreated Containers		All Containers	
	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.
<1	93	24.7	..	..	362	6.8	562	6.8
Treatment [1]								
+5	93	2.2	238	0	127	2.4	365	0.8
+9	91	3.3	259	0	400	0.8	659	0.5
+13	92	3.3	79	0	228	1.3	307	1.0
+17	93	11.8	103	0	437	2.7	540	2.2
+21	91	18.7	188	1.1	469	3.8	657	2.7

[1] April 29–May 3, 1968.

of Gardona was applied to the premises of the two municipios. One-third of this amount represented the 2.5 percent formulation, the balance 1.25 percent. In urban Corozal each two-man team applying the insecticide with hand sprayers averaged five premises per 5-hour day, whereas in urban Naranjito the average was 6.8 premises. Coverage of the rural premises was much slower, the average ranging between 2.0 and 2.2 premises. Treatment with power equipment involved 6,450 gallons of Gardona spray, with 62 percent being applied in urban Corozal.

The data for cholinesterase values of 10 spraymen, two supervisors, two packagers and controls are listed in Table 7. All values were considered to be in the normal range of variation except for those of sprayman number 10 and the two packagers on April 19. On April 24 these men were assigned to duties not exposing them to Gardona or other insecticides. However, samples from the same individuals on April 25 indicated normal values for each; consequently, the men returned to their regular duties and subsequently none of those individuals showed any abnormal values. Since the recovery to normal levels was more rapid than would normally be expected, it was concluded that the low readings obtained with the April 19 samples were probably due to accidental contamination of the sampling tubes with

TABLE 6.—Number of containers inspected in three municipios and percentage of marked and unmarked (untreated) containers detected on each survey.

Area	Weeks after Treatment	Total	Percent Marked	Percent Unmarked
Urban Corozal	5	48,771	58	42
	10	45,151	52	48
Rural Corozal	5	4,222	59	41
	10	4,101	40	60
Urban Naranjito	5	18,570	53	47
	9	19,046	34	66
	13	16,122	22	79
Rural Naranjito	5	6,505	57	43
	9	5,242	40	60
	13	5,751	28	72
Urban [1] Morovis	0	26,035	100	..
	5	21,596	65	35
	10	25,987	53	47

[1] Untreated town in which containers were marked at time of first inspection.

TABLE 7.—Cholinesterase levels[1] in persons spraying Gardona suspension (1.25 and 2.5%) packaging 75% Gardona wettable powder, and in supervising spraymen.

		Preexposure		Exposure				
		Mar. 8	Mar. 29	Apr. 5	Apr. 19	Apr. 25	May 10	May 24
Spraymen								
1	RBC	11.9	14.4	12.3	10.8	..	20.0	13.4
	Plasma	4.0	4.3	3.5	3.6	..	6.5	2.4
2	RBC	12.9	15.5	13.2	10.8	..	22.0	14.0
	Plasma	6.8	4.8	5.3	4.7	..	5.7	4.7
3	RBC	12.6	13.9	..	12.6	..	21.0	16.8
	Plasma	5.5	4.9	..	5.1	..	6.4	4.5
4	RBC	11.2	12.8	12.1	..	..	..	15.4
	Plasma	4.9	4.9	4.4	..	..	..	4.0
5	RBC	11.7	15.5	14.6	13.7	..	18.2	13.7
	Plasma	6.4	4.9	5.5	5.5	..	4.7	5.4
6	RBC	11.2	13.4	11.6	9.1	..	20.0	16.5
	Plasma	4.1	4.8	3.8	3.9	..	5.2	3.2
7	RBC	13.3	13.9	..	12.3	14.9	19.6	14.1
	Plasma	4.7	4.8	..	4.1	4.7	4.9	3.2
8	RBC	12.2	10.7	13.1	10.0	..	18.0	14.6
	Plasma	5.0	4.4	4.4	4.7	..	5.1	2.7
9	RBC	13.4	13.9	13.0	13.8	14.9	20.0	14.7
	Plasma	4.8	4.8	3.9	4.0	4.4	4.6	2.7
10	RBC	..	13.9	..	4.8[3]	17.6	19.4	14.3
	Plasma	..	4.1	..	3.3	3.7	5.3	3.0
Packagers								
1	RBC	..	16.6	12.4	5.4[3]	18.0	22.0	15.6
	Plasma	..	4.8	3.4	1.8	3.9	5.0	2.0
2	RBC	..	..	12.2[2]	7.0[3]	14.0	13.6	14.4
	Plasma	..	..	5.3[2]	3.8	5.6	3.8	3.8
Spray Supervisors								
1	RBC	13.0	14.4	11.7	13.2	..	17.5	13.6
	Plasma	4.7	4.8	4.4	4.6	..	6.2	3.6
2	RBC	15.0	15.0	13.0	16.4	..	22.0	15.6
	Plasma	5.3	4.9	4.1	4.6	..	5.0	2.0
Controls								
1	RBC	..	16.0	12.3	10.8	15.0	15.8	13.4
	Plasma	..	4.7	4.2	4.4	4.6	5.9	4.4
2	RBC	..	..	12.2	13.6	15.9	18.0	13.4
	Plasma	..	..	5.3	5.3	6.2	6.7	5.2

[1] Values reported are expressed in micromoles acetylcholine hydrolyzed/minute/ml. plasma or packed red blood cells.

[2] Blood samples taken on April 15.

[3] These low values were considered probably to be due to accidental contamination of the sampling tubes with Gardona.

Gardona. The data for the personnel engaged in these operations with Gardona thus indicate that the insecticide can be safely applied as a premises treatment.

SECOND SPRAY CYCLE

From the results achieved in the first spray cycle, decision was made to re-treat urban Corozal and Naranjito with a 2.5 percent Gardona suspension, to continue inspection but not re-treat rural Corozal and Naranjito, and to spray Morovis with 2.5 percent Gardona suspension.

Municipio Corozal: During weeks 35 to 38, urban Corozal was re-treated using a 2.5 percent Gardona suspension with the same techniques followed in the first spray cycle. The index for containers positive for *Ae. aegypti* larvae at the time of treatment was 1.1 (see Table 1). At 4, 8 and 12 weeks after the second application, the indices in treated containers were 0.03, 0.03 and 0.3 percent (Table 8). In comparison, new untreated containers showed indices of 0.6, 0.6 and 1.8 percent for the same successive time intervals.

Ovitrap data for 75 units indicated approximately 30 percent positivity for each of the 3 weeks preceding treatment. The indices for positive ovitraps during the treatment period (weeks 35–38) were 37, 29, 21 and 20. For weeks 39 through 48 the indices ranged from 1.3 to 8.1.

Municipio Naranjito: Urban Naranjito was re-treated on weeks 38–39. The previous index of this community showed 0.3 percent of the containers to be positive for larvae of *Ae. aegypti* (Table 4). After re-treatment, indices of *Ae. aegypti* larvae ranged between 0 and 0.04 percent through week 17 (Table 9). On week 22 the index was 0.13 percent. In the unmarked containers the indices for weeks 4 and 8 were 0.08 and 0.09; for weeks 12 and 17, 0.25 and 0.35; and for week 22, 0.59 percent.

The percentages of the 45 ovitraps positive for eggs of *Ae. aegypti* for 3 weeks prior to treatment were 6.8, 4.4 and 4.4. On the first week of treatment (week 38) the index rose to 18.2 percent, but for the subsequent weeks 39 to 47 the maximum percentage of positive traps was 2.4.

Municipio Morovis: This untreated

TABLE 8.—Percent of premises and wet containers positive for *Aedes aegypti* larvae in urban Corozal, second spray cycle.

Inspection (weeks after treatment) [1]	Premises		Treated Containers		Untreated Containers		All Containers	
	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.
4	1283	0.70	2770	0.03	1322	0.60	4092	0.21
8	1288	0.69	2642	0.03	1671	0.59	4313	0.25
12	1290	4.03	4971	0.30	3596	1.77	8567	0.92

[1] Re-treated August 28–September 21, 1968. Index before re-treatment was 1.1 (Table 1).

TABLE 9.—Percent of premises and wet containers positive for *Aedes aegypti* larvae in urban Naranjito, second spray cycle, with 2.5 percent Gardona suspension.

Inspection (weeks after treatment) [1]	Premises		Treated Containers		Untreated Containers		All Containers	
	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.
4	785	0.12	2559	0	1237	0.08	3796	0.02
8	787	0.25	2332	0.04	1031	0.09	3363	0.05
12	770	0.51	2849	0	1545	0.25	4394	0.09
17	780	0.76	2118	0	1981	0.35	4099	0.17
22	794	0.37	1503	0.13	1505	0.59	3008	0.36

[1] Re-treated September 19–28, 1968. Index before re-treatment was 0.3 (Table 4).

community was sprayed during weeks 34 and 35. A survey on weeks 32–33 revealed that 18.3 percent of the 3,554 containers inspected were positive for *Ae. aegypti* larvae (Table 10). In inspections

In urban Naranjito the percent reduction at week 13 was not as favorable as in Corozal, either in relation to original pretreatment index or to that for the untreated containers. The significance of

TABLE 10.—Percent of premises and wet containers positive for *Aedes aegypti* in urban Morovis after treatment with 2.5 percent Gardona suspension.

Inspection (weeks)	Premises		Treated Containers		Untreated Containers		All Containers	
	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.	No. Insp.	% Pos.
−2	830	28.91	..	..	3554	18.31	..	..
Treatment [1]								
+4	815	3.68	3615	0.08	1818	1.76	5433	0.64
+8	824	5.09	3348	0.11	2194	2.09	5542	0.90
+12	835	4.79	2584	0.23	2355	1.78	4939	0.97
+16	817	4.03	2644	0.49	2336	3.98	4980	2.12

[1] August 19–30, 1968.

on weeks 4, 8, 12 and 16 after the application of Gardona, the indices in wet containers were 0.08, 0.11, 0.23, and 0.49. Unmarked containers showed indices in wet containers of 1.8, 2.1, 1.8 and 4.0 for the same successive intervals.

In the 4 weeks prior to treatment indices for ovitraps positive for *Ae. aegypti* eggs ranged between 43.2 and 47.2 percent. During the 2 weeks of treatment the indices were 48.6 and 16.7 percent. In the indices for the next 12 weeks, the percentage of ovitraps positive for eggs of *Ae. aegypti* ranged between 2.9 and 14.7, but no consistent trends were apparent.

DISCUSSION

The data from these studies establish that Gardona applications of 1.25 or 2.5 percent suspensions provided marked reduction of larval indices of *Ae. aegypti* in treated containers in both rural and urban areas. In urban Corozal (2.5 percent suspension) the results of the inspection on the 10th week represented an 82 percent reduction when compared to the pretreatment indices. In relation to the index for the untreated containers, the reduction was 77 percent. Container indices for larval infestations in untreated Morovis were 19 times those of treated Corozal.

these findings is lessened considerably because of the relatively low level of infestation (1.1–1.5 percent) before treatment. Nonetheless, the absence of any breeding for 9 weeks after treatment was encouraging, and the validity of this view is strengthened greatly when the data for Barrio Guadiana (Table 5) are reviewed. This area, with a pretreatment level equal to urban Corozal (6.8 percent) and much higher than that for urban Naranjito, showed a complete absence of treated containers positive for *Ae. aegypti* larvae on the inspection 17 weeks after it was sprayed with 2.5 percent suspension. In contrast, the untreated containers had indices of 0.8 to 2.7 percent. In Barrio Palmarejo sprayed with 1.25 percent Gardona suspension, few infested containers were found 21 weeks after treatment, but here the pretreatment level was only 1.9 percent.

Re-treatment of urban Corozal and Naranjito with 2.5 percent Gardona suspension at times when the indices were low from the first cycle treatment showed that the applications essentially eliminated further breeding in the treated containers for 8 and 17 weeks, respectively. Moreover, treatment of Morovis (the untreated urban area for the first spray cycle) with 2.5 percent Gardona at a time when the

index was 18.3 percent demonstrated the high level of effectiveness of the Gardona application when a 97 percent reduction was present 16 weeks after application. Untreated containers showed an infestation rate of about 4.0 percent, approximately eight times that of the treated receptacles.

The data for all applications indicate that Gardona was highly effective in controlling or preventing the breeding of *Ae. aegypti* larvae in containers. Although the 1.25 percent formulation performed particularly well in Barrio Palmarejo, the results in urban Corozal and Morovis suggest that the 2.5 percent concentration would be the choice for control operations. The absence of adverse responses in the cholinesterase levels of the spraymen and other personnel handling Gardona supports the view that its use does not offer any marked hazard to man. Since the acute oral LD-50 dosage for male and female rats is greater than 4,000 mg./kg., these results were not unexpected (Gaines, 1969).

The results also suggest that premises applications based on 2, 3 or 4 monthly intervals are not sufficiently productive to achieve the ultimate aim of the program, the eradication of the species. Table 6 clearly shows that the rate at which new untreated containers accumulate in a community is so rapid that the mosquito population has numerous untreated sites in which to oviposit. In treated urban Corozal and Naranjito, the percentages of unmarked containers positive for larvae were 7 to 12 percent higher than in untreated Morovis, on the 5-week survey. This fact suggests that the insecticide treatment possibly might repel the female, thereby causing her to seek an untreated site for oviposition. The data at week 10 show little difference in the percentage of unmarked positive cans in Corozal and Morovis, but a 19 percent difference exists between Morovis and Naranjito. These findings do not discount the possible factor of repellency of the treated receptacles, since the containers in Naranjito were sprayed with 2.5 percent Gardona and theoretically could be less attractive to ovipositing females than those in Corozal treated with the 1.25 percent formulation. Such repellency has been reported from studies with insecticidal treatment of houses where the residues repelled or deterred anopheline mosquitoes from entering. Since the degree of repellency may differ with the insecticide used, this factor may also vary in its importance. In the Florida studies (Eliason and von Windeguth, unpublished manuscript) the *Ae. aegypti* larval indices (18.7 and 29.6 percent) of the treated wet containers were greater than those of the untreated containers (*i.e.*, 12.3 and 19.2 percent) in the same premises during the 8th and 12th week posttreatment inspections. In contrast, the Gardona-treated areas showed indices in wet containers of 1.3 and 1.6 percent versus 16.9 and 16.5 percent for the same time intervals. Such findings might suggest lack of repellency to malathion, but they definitely show a lack of effectiveness in controlling the species.

One of the critical factors in *Ae. aegypti* control in small containers is the rapidity with which new receptacles appear on premises. Von Windeguth *et al.* (1969) reported that during a 3-month period, the total number of containers in an urban area of nine blocks remained essentially the same. However, in the various blocks the percent of marked containers removed during that period ranged from 43 to 68 and averaged 55 for the nine-block group. Over the same period 53 percent of the containers found were new and unsprayed.

This rapid turnover in containers offers a serious obstacle to successful eradication procedures. The concept of an insecticide that would remain effective for 2 to 5 months loses its significance when new untreated containers are available for the species to continue its propagation. In addition, the ovitrap data from these tests suggest that the adult female mosquitoes present during the time of treatment continue to lay eggs for their life-span which probably extends beyond the period of treatment. Because of this condition, the possibility of repellency of treated contain-

ers to the adult female, and the rapid appearance of untreated containers, the chances of drastically and immediately curbing *Ae. aegypti* populations by premises treatment alone decline greatly. A partial solution to this problem would be the use of adulticiding measures simultaneously with the premises application so that adults would not be present to deposit their eggs in the new untreated containers that appeared in the area. Such adulticiding applications might well be required at monthly or less frequent intervals to insure that no ovipositing females would be present.

The necessity for frequent chemical operations focuses the attention again on the fact that sanitational measures to curb the indiscriminate disposal of these containers on premises or to remove such receptacles might prove the most feasible and effective eradication measure. Since this type of procedure *per se* is frequently a difficult practice to enforce, the operational approach that appears to be most feasible would be to use a combination of sanitational and chemical measures that would be followed within a given area until the biological data indicated successful eradication. Once the latter is achieved, the control activities would advance to adjacent areas, and the original operational area would be placed under surveillance only.

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