LABORATORY AND FIELD ASSESSMENT OF INSECT GROWTH REGULATORS FOR MOSQUITO CONTROL [1]

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ABSTRACT. Of 65 IGR compounds tested in the laboratory with 4th-stage larvae of *Anopheles quadrimaculatus* Say, 5 had LC-90 values of less than 0.020 ppm, 14 were below 0.165 ppm, and 46 others were greater than 0.210 ppm. Four of 8 formulated IGRs tested in small field plots were effective at rates of 0.01 to 0.05 lb/acre (11 to 56 g/ha). Two of the most promising compounds, methoprene (isopropyl 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate 57% (*E,E*)- and 33% (*Z,E*) and Dimilin® (*N*-(4-chlorophenyl)-*N'*-(2,6-difluorobenzoyl)urea), were effective at rates of 0.025 and 0.02 lb/acre (28 and 22 g/ha), respectively, against natural populations of *Culex nigripalpus* Theobald and *Cx. salinarius* Coquillett. Also, in salt-marsh mangrove habitats, *Aedes taeniorhynchus*. (Wiedemann) was completely controlled when 0.025 lb of Dimilin® or 0.05 lb of methoprene were applied by helicopter in 5 to 10 gal (19 to 39 liters) of aqueous formulation/acre. Insecticide-resistant strains of *Ae. taeniorhynchus* and *An. quadrimaculatus* were as susceptible to these 2 compounds as were nonresistant strains of the same species.

One of the more promising uses for insect growth regulators (IGR) is the control of aquatic stages of mosquitoes. However, synthetic juvenile hormone analogues and other types of compounds that affect development may also modify behavior of adult mosquitoes that survive larval or pupal exposures to IGRs. Also, the IGRs could provide an alternative source of control for the several species of mosquitoes that have developed high levels of resistance to conventional insecticides. The value of these compounds for control of immature stages has been considered by a number of workers (Schaefer and Wilder 1972, 1973; Schaefer and Dupras 1973; Schaefer et al. 1974a, 1974b, 1975; Jakob and Schoof 1971, 1972; Jakob 1972; Steelman and Schilling 1972; Steelman et al. 1975; Hsieh and Steelman 1974; Mulla et al. 1974, 1975; Hoppe et al. 1974). We report here the results we obtained when we examined the effectiveness of 65 of these compounds in the Gainesville laboratory on larvae of *Anopheles quadrimaculatus* Say. In addition, we conducted small-plot field tests with those compounds that were most effective in the laboratory and that were available. The most promising were field tested in Lee County where they were applied by helicopter against natural populations of the black saltmarsh mosquito *Aedes taeniorhynchus* (Wiedemann).

MATERIALS AND METHODS

LABORATORY SCREENING. The laboratory assays were conducted in 18x28-cm enamel pans containing 1 liter of distilled water. First, known concentrations of the candidate compounds were prepared by pipetting 0.1 to 10 ml of stock solutions of as much as 1 mg of active ingredient/ml in acetone or water into the distilled water and then stirring to assure uniform dispersion. Then 50 early 4th-stage larvae obtained from a laboratory colony of an insecticide-susceptible strain of *An. quadrimaculatus* were placed in each pan, and larval food was added immediately (Ground Hog Supplement, 40% protein;

[1] This paper reflects the results of research only. Mention of a pesticide in this paper does not constitute a recommendation for use by the USDA nor does it imply registration under FIFRA as amended.

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Purina Food Corp.) and again daily or as required. The untreated control for each day's assay was one or more pans of distilled water handled in the same way except without the active ingredient. The test pans were held in a room maintained at 27 to 29° C and 65 to 75% relative humidity. Each day all newly formed pupae were counted, removed from the treated water, rinsed with distilled water, and transferred to a container of distilled water in a 25x25x16 cm cage for observation of eclosion. The dead larvae were likewise counted, removed from the pans, and discarded. Two or more days after the last pupa was collected from each pan, the percentage eclosion was recorded. The data thus provided information about concentrations that caused larval mortality and about those that prevented successful emergence after pupation. However, for analysis the information was combined and summarized as the number of adults that emerged successfully from the 50 larvae.

The initial testing was done with 3 to 5 concentrations of each compound to determine the general level of activity. Subsequently, a minimum of 3 concentrations giving between 0 and 100% mortality was replicated to obtain the data necessary to establish a dosage-response relationship. However, in those instances in which the compound was ineffective, the second series of 3 or more concentrations included only 1 previously used concentration to confirm the original result. In the same way we tested the effectiveness of 2 IGRs against DDT(1,1, 1-trichloro-2,2-bis(*p*-chlorophenyl)ethane)-resistant (Hartwell Dam) and susceptible strains of *An. quadrimaculatus* and malathion (diethyl mercaptosuccinate *S*-ester with *O,O*-dimethyl phosphorodithioate)-resistant (Duval) and susceptible strains of *Ae. taeniorhynchus*. The data were adjusted by Abbott's formula, converted to log-probits, and analyzed with a programmable calculator to establish the concentration required to reduce eclosion by 50% (LC-50) and 90% (LC-90) with slope and correlation estimates; data from 350 to 1000 specimens were used for these calculations.

Where a compound was sufficiently promising, the survivors of the test concentrations were given an opportunity to mate, and the females were offered a blood meal and later transferred to individual vials for oviposition. The resulting eggs were allowed sufficient time to mature, and the percentage hatch was determined.

SMALL-PLOT FIELD TESTS. For the small-plot field tests of promising compounds, artificial ponds were constructed outdoors by excavating the soil to a depth of about 2.5 ft (0.8 m), lining the hole with polyethylene sheeting, and then covering the sheeting with centipede or St. Augustine sod. The resulting plots ranged from 40 to 60 ft^2 (3.7 to 5.6 m^2) and each was flooded to a depth of 15 cm on the day before testing. Pretreatment samples of the water were taken routinely to confirm that the plot was uncontaminated (with residues of insecticides) at the time of treatment; however, on a few occasions the bioassays indicated some residual activity from previous treatments. In these cases, the data were discarded, and the test was rerun.

Treatments were applied to the plots manually by pipetting or by pouring into each pond an aqueous solution containing the required amount of the test compound; the water was then stirred to assure thorough and uniform dispersion. Two hr after treatment 2 liters of water were removed from each pond and transferred to the laboratory. There gross foreign matter was removed by filtering through organdy or cheesecloth, and a 1-liter aliquot was placed in the standard 18x30-cm enamel pan. The testing procedure thereafter was the same as that described for the screening tests and was initiated with the introduction of fifty 4th-stage *An. quadrimaculatus* larvae into each pan. These tests were replicated, and separate untreated control plots were assayed with each replication. Further observations were made with water samples at 24 and 48 hr posttreatment.

The outdoor ponds were also used in tests of indigenous populations of mosquitoes that occurred as a result of natural or artificial flooding. Applications were made in the same manner as described for the previous test. The relative numbers of the various species present were determined by collecting 4th-stage larvae from the ponds and identifying them. Assays of pupal mortality were made by collecting about 50 pupae from each pond each day and observing emergence in the laboratory. Since the populations were cycling naturally, on some days there were few or no pupae present; then 50 random dips were made in each pond, and the pupae recovered were considered the sample.

AERIAL APPLICATIONS. Aerial applications of Dimilin® (*N*-(4-chlorophenyl)-*N'*-(2,6-difluorobenzoyl)urea), a 25% WP formulation of Compound I and methoprene (isopropyl 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate 57% (*E,E*) and 33% (*Z,E*) as Altosid SR-10®, a 10% slow release formulation of Compound IV) were made during the summer in Lee County near St. James City (4-acre plots) and on Galt Island (one 30- and one 15-acre plot) and Sanibel Island (one 100- and two 10-acre plots) where salt-marsh mosquitoes, mostly *Ae. taeniorhynchus,* were breeding in flooded mangroves and adjacent grasslands. The applications were made from a helicopter fitted with 23 to 24 flat fan nozzles (Spraying Systems No. 8010) calibrated at 50 psi (3.5 kg/cm^2) to deliver 30 gal (114 liters)/min. The aircraft was flown just above the treetops at 60 mph (97 km) utilizing 50 ft (15 m) measured swaths. However, when the plots exceeded 45 acres (18 ha), the pilot judged the swaths by visual observation of landmarks rather than by swath markers. Only 2 dosages of a given compound were tested on any given day, a minimum dose and a maximum dose that was double the minimum dose. The maximum dosage was applied by making 2 passes over each swath; the lower dosage was applied by making only 1 pass. Thus, in some tests, 10 gal (38 liters) of aqueous formulation and in others 5 gal (19 liters) were used to obtain the desired dosage of active ingredient.

The treatments were made when the larvae had reached the 3rd-stage. Effectiveness of the compounds was assessed (1) by recording the number of live and dead or moribund larvae, and the number of live and dead pupae and exuviae in samples of 100 or more specimens, or, when 100 were not available, all specimens in 50 dips; and (2) by collecting 50 to 100 pupae at each of a minimum of 2 sampling locations within each treatment area and placing them in 25x25x16-cm aluminum screened cages that were partially immersed in the breeding water at the collection site. Untreated control plots were assayed in each replication.

RESULTS AND DISCUSSION

LABORATORY SCREENING. The results obtained in the screening tests with 20 of the 65 tested compounds are given in Table 1 (compounds listed in order of decreasing activity). The remaining 45 compounds were less effective with LC-90 values greater than 0.215 ppm; the results are available to interested persons on request. Compounds I-IV were outstanding in ability to reduce eclosion and were similar in toxicity levels in the laboratory to most of the better larvicides currently available for mosquito control. Also, Dimilin (Compound I) and TH-6038 (Compound II) produced extensive mortality in larvae at levels exceeding the LC-50 range which was based on adult eclosion. Compounds III and IV were not efficient larvicides but were active in preventing adult eclosion. Compounds V-VIII were also effective, since the LC-90 levels ranged between 0.019 and 0.034 ppm, while IX-XV had LC-90 values of less than 0.1 ppm. None of the compounds delayed larval development, and none of those tested for effect on reproduction were found to reduce fertility.

Since our laboratory tests and those of others indicated that TH-6040 and metho-

Table 1. IGRs most effective in the laboratory against 4th stage larvae of *Anopheles quadrimaculatus* (Results with 45 less effective compounds available on request).

Compound	Company designation or common name	Chemical name	Slope	LC-90 (ppm)	Correlation coefficient (r)
I	Thompson-Hayward TH-6040	1-(*p*-chlorophenyl)-3-(2,6-difluorobenzoyl)urea	6.24	0.004	.79
II	Thompson-Hayward TH-6038	1-(*p*-chlorophenyl)-3-(2,6-dichlorobenzoyl)urea	5.47	0.008	.69
III	Chevron Ortho-17565	*S*-benzyl 3,5-di-*tert*-butyl-4-hydroxythiobenzoate	1.39	0.009[a]	.47
IV	Zoecon ZR-0515 methoprene	Isopropyl 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate 57% (*E,E*)- and 33% (*Z,E*)	2.63	0.011	.84
V	Chevron Ortho-18286	*S*-*tert*-butyl 3,5-di-*tert*-butyl-4-hydroxythiobenzoate	1.89	0.019[a]	.85
VI	Zoecon ZR-0338	(*E*)-4[(6,7-epoxy-3,7-dimethyl-2-octenyl)oxy]-1,2-(methylenedioxy) benzene	1.49	0.020	.63
VII	Hoffman-LaRoche RO-7-9767	4-[(6,7-epoxy-3-ethyl-7-methyl-2-nonenyl)oxy]-1,2-(methylenedioxy)benzene	3.34	0.023	.75
VIII	Stauffer R-31026	*S,S'*-diisobutyl ethylethylenebis = [thiocarbamate]	3.69	0.034	.89
IX	AEQI[b] AI3-34601	4-[(6,7-epoxy-3,7-dimethyl-2-nonethyl)oxy]-1,2-(methylenedioxy)benzene	2.25	0.073	.74
X	Zoecon ZR-0340	(*E*)-4-[(7-ethyl-3-methyl-2,6-nonadienyl)oxy]-1,2-(methylenedioxy)benzene	1.91	0.079	.72
XI	Zoecon ZR-0337	(*E*)-4-[(7-ethoxy-3,7-dimethyl-2-octenyl)oxy]-2,2-(methylenedioxy)benzene	2.17	0.092	.91
XII	Chevron Ortho-17937	2,6-di-*tert*-butyl-4-[(trichlorovinyl) thio]phenol	1.70	0.095[c]	.71
XIII	Hoffman LaRoche RO-8-5497	10,11-epoxy-3,7,11-trimethyl-1-(2-propynyloxy)-2,6-tridecadiene	2.35	0.095	.87
XIV	Hoffman LaRoche RO-20-3600	(*E*)-4-[(6,7-epoxy-3,7-dimethyl-2-nonenyl)oxy]-1,2-methylenedioxy)benzene	2.91	0.099	.96
XV	Monsanto Mon-0585	2,6-di-*tert*-butyl-4-(α,α-dimethylbenzyl)phenol	3.31	0.099	.89
XVI	Zoecon ZR-0172	(+)-4-[(6,7-epoxy-3,7-dimethyloctyl)oxy]-1,2-(methylenedioxy)benzene	2.73	0.151	.77
XVII	AEQI[b] AI3-34308	phenyl (6,7-epoxy-3,7-dimethyloctyl) carbamate	1.33	0.156	.80
XVIII	AEQI[b] AI3-34934	4-[(6,7-epoxy-3,7-dimethyl-octyl)oxy]-1,2-(methylenedioxy)benzene	2.22	0.161	.99
XIX	Stauffer R-20458	(*E*)-6,7-epoxy-1-(*p*-ethylphenoxy)-3,7-dimethyl-2-octene	2.05	0.163	.82
XX	AEQI[b] AI3-34592	(*E*)-6,7-epoxy-1-(*p*-cumenyloxy)-3,7-dimethyl-2-octene	3.21	0.215	.57

[a] Formulated material, 50% WP.
[b] Agricultural Environmental Quality Institute, USDA.
[c] Formulated material, 4 lb/gal (0.48 kg/liter) EC.

prene were likely candidates for mosquito control, we conducted assays to determine the susceptibility of insecticide-resistant strains of *An. quadrimaculatus* and *Ae. taeniorhynchus* to each of these compounds. The results (Table 2) showed no substantial differences in the susceptibility of the resistant and susceptible strains to either compound. Thus, natural populations of *Ae. taeniorhynchus* that have developed resistance to malathion, e.g., those in Florida, are not likely to develop an immediate tolerance to either of these 2 compounds. Nevertheless, the effect of repeated exposure of large populations of *Ae. taeniorhynchus* on the development of resistance cannot presently be predicted. However, resistance to IGRs has been artificially induced in laboratory strains of *Culex tarsalis* Coquillett (Georghiou et al. 1974) and *Cx. pipiens quinquefasciatus* Say (Brown and Brown 1974).

Table 2. Effect of IGRs on 4th stage larvae of susceptible and resistant strains of *Anopheles quadrimaculatus* and *Aedes taeniorhynchus*.

Species	Strain	LC-50 (ppm)	LC-90 (ppm)
	Compound I, TH-6040		
quadrimaculatus	DDT-susceptible	0.003	0.004
	DDT-resistant	0.002	0.003
taeniorhynchus	malathion-susceptible	0.001	0.002
	malathion-resistant	0.001	0.003
	Compound IV, methoprene		
quadrimaculatus	DDT-susceptible	0.004	0.011
	DDT-resistant	0.004	0.015
taeniorhynchus	malathion-susceptible	0.0002	0.002
	malathion-resistant	0.0002	0.001

SMALL-PILOT FIELD TESTS. The results obtained when 9 of the top 20 compounds were added to artificial ponds and the water was bioassayed at 2 hr posttreatment are given in Table 3. Compounds I-IV applied at a rate of 0.05 lb/acre gave 100% control of eclosion and compounds II and IV applied at a rate of 0.01 lb/acre gave 99% control. Compound XV was 100% effective at 0.2 lb/acre, and the other materials were highly or completely effective at 0.5 or 1 lb/acre (560 or 1121 g/ha); however, at 0.1 lb/acre (112 g/ha), none of these provided adequate control.

Most of the compounds tested in these field ponds were inactive after 24 hr at practical dosage levels of 0.1 lb/acre (112 g/ha.) or less, but some special formulations did prolong or increase effectiveness. For example, the slow release formulation of methoprene was more effective after 24 and 48 hr than the flowable and EC formulations. However, the slow release formulation of Compound XIX did not give better results 24 and 48 hr after treatment than did the 4 lb/gal (0.48 kg/liter) EC. At 48 hr, 1 lb/acre of the 5% EC formulation of TH-6040 completely controlled eclosion, while only 0.05 lb/acre of the 25% WP formulation achieved the same result. Furthermore, at 24 hr posttreatment, 0.01 lb/acre of the WP eclosion was 59% compared with 88% with the EC formulation; however, with the WP all emerged adults died on the water surface, but with the EC all that eclosed lived. (Morphological deformities were common in adults emerging from sublethal exposures to the WP formulation.)

Many of the IGRs are effective primarily against 4th-stage larvae and therefore must be available in the water when the larvae reach this stage. However, in practical situations it is usually impossible to treat only 4th-stage larvae. Therefore, the material must have some residual activity to assure that the 4th-stage larvae

Table 3. Effectiveness of IGR formulations in small field plots determined by laboratory bioassay of water samples at 2 hr posttreatment with 4th stage larvae of *Anopheles quadrimaculatus* (means of 2–6 replications).

Compound & Company desig.	Formulation	% Control of eclosion[a] with indicated concentration (lb/acre; g/ha in parentheses)								
		0.005 (5.6)	0.01 (11.2)	0.025 (28)	0.05 (56)	0.1 (112)	0.2 (224)	0.25 (280)	0.5 (560)	1.0 (1121)
I TH-6040	5% EC	...	81	...	100	100	...	...	...	...
	25% WP	73	83	100	100	...	...	...	...	...
II TH-6038	10%EC	73	99	...	100	...	...	...	...	...
III Ortho 17565	50% WP	...	51	...	100	100	...	...	...	...
IV ZR-0515	4 lb/gal EC[b]	...	82	...	94	100	...	...	...	...
	10% flowable	...	78[c]	...	100	100	...	...	...	...
	10% slow release	...	99	...	98	100	...	...	...	...
VII RO-7-9767	25% EC	...	...	...	...	19[c]	66	...	98	100
XIII RO-8-5497	4 lb/gal EC[b]	...	...	...	...	61	...	...	96	100
XIV RO-20-3600	4 lb/gal EC[b]	...	...	...	...	19[c]	37	...	98	100
XV MON-0585	3 lb/gal EC[b]	...	...	...	...	45[c]	100	...	99	100
XIX R-20458	4 lb/gal EC[b]	...	...	...	...	58	70	...	99	100
	2 lb/gal slow release[b]	...	49	...	67	72	...	76	100	...

[a] Eclosion in untreated controls: 90% (range 80–92%).
[b] 4 lb/gal (0.48 kg/liter); 3 lb/gal (0.36 kg/liter); 2 lb/gal (0.24 kg/liter).
[c] 1 replicate.

will have adequate exposure, even when the 3rd or earlier instars are treated or when populations of mixed ages are treated. While this factor creates a need for special formulations that prolong IGR activity under field conditions, the inherent instability of most of these compounds is beneficial because it limits the persistence of most IGR materials.

The results of tests with TH-6040 and methoprene against mixed natural populations in the small field ponds are reviewed in Table 4. These populations were predominantly *Cx. nigripalpus* Theobald (64%) and *Cx. salinarius* Coquillett (26%) though there were some *Cx. p. quinquefasciatus* (4%), *An. quadrimaculatus* (3%), and *Psorophora columbiae* (Dyar & Knab) (3%). Water in the plots ranged from 6.4 to 7.3 in pH and from 20 to 36° C. Neither compound was completely effective against larvae that were about to pupate or pupae collected on the first day after treatment. However, Dimilin at 0.01 to 0.04 lb/acre (11–45 g/ha) was an effective larvicide and caused an extensive reduction in the population of immature stages the day after treatment (Table 4). Indeed, very few pupae were found on days 2 and 3 in any pond and through day 6 in ponds treated at rates of 0.02 and 0.04 lb/acre. With methoprene applied at higher rates, the trend was similar but the effect on the larvae was not as extreme. The major effect of the application of methoprene was the total prevention of eclosion by those pupae formed after exposure for 2–3 days to rates of 0.025 to 0.1 lb/acre. At the lower rates, the effect lasted through day 8; at the highest rate it lasted through day 12. Both methoprene and TH-6040 were therefore highly effective and provided greater than 90% control by the second day after all treatments; 100% control was achieved by day 3 with 0.04 lb/acre of TH-6040 and 0.05 lb/acre of methoprene and by day 4 with 0.025 lb/acre of methoprene. The slow release formulation of methoprene provided control for a longer duration than did TH-6040.

AERIAL APPLICATIONS. The aerial applications of methoprene and Dimilin to areas with light to medium density of mangrove growth provided information about the dosages required to control natural populations of *Ae. taeniorhynchus* (Table 5). In the 4-acre (1.6 ha) plots, 0.1 and 0.05 lb/acre of methoprene and Dimilin, respectively, were required for effective control in the first replication. However, observations along the perimeters of the test plots indicated that some of the materials had drifted further than anticipated during application thereby leaving some of the upwind areas with light coverage. In the second replication, rates of 0.05 and 0.025 lb/acre of methoprene and Dimilin, respectively, provided complete control. Also at 0.025 lb/acre Dimilin caused complete or high initial larval mortality, and when the initial larval mortality was incomplete, numerous dead pupae and adults were observed on the water surface 48 to 72 hr after treatment. In fact, at 72 hr, some areas of the plot treated with 0.025 lb/acre where drift apparently occurred had many live adults on the water surface that were visibly crippled and were unable to fly. This phenomenon was not observed with sublethal applications of methoprene.

Casual observations of other aquatic species and spiders and spot checks of nearby apiaries revealed no obvious immediate effect of either Dimilin or methoprene on nontarget organisms. This topic is discussed at length by several authors (Miura and Takahashi 1973, 1974, 1975; Steelman and Schilling 1972; Steelman et al. 1975; Mulla et al. 1975; Norland and Mulla 1975; Schaefer et al. 1974a).

When larger plots were treated, both methoprene and Dimilin were again highly effective. The first test with methoprene was conducted on Sanibel Island where 0.025 lb/acre was applied to 100 acres (40 ha) and 0.05 lb/acre was applied to 10 acres (4 ha) near Wulfert's Point. The treated areas were primarily flooded mangrove habitat but some portions had heavy canopy, and others were fairly open. Immediately after the applications, heavy rains flooded the area on

Table 4. Effect of TH-6040 (Dimilin) and methroprene (Altosid SR-10) on natural populations of mosquitoes, predominantly *Culex* species, in small field plots (means of 2 replications).

Days after treatment	Untreated control		Mean number of pupae and % eclosion at indicated concentration (g/ha in parentheses)											
			TH-6040 (Dimilin)						Methoprene (Altosid SR-10)					
			0.01	(11.2)	0.02	(22)	0.04	(45)	0.025	(28)	0.05	(56)	0.1	(112)
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	35	100	50	86	27	86	30	92	38	34	27	50	27	28
2	14	97	5	41[a]	2	20[a]	1	50[a]	23	12[a]	17	50	25	58
3	22	96	8	30[a]	2	25[a]	0		50	2[a]	6	0[b]	14	0[b]
4	15	94	20	64	0.5	50[a]	0		50	0[b]	2	0[b]	4	0[b]
5	13	100	24	88	1	50[a]	0.5	50[a]	18	0[b]	6	0[b]	5	0[b]
6	13	100	50	89	6	100	0		20	0[b]	4	0[b]	26	0[b]
7	13	95	48	88	28	100	14	50	26	0[b]	6	0[b]	35	0[b,c]
8	12	100	34	82	26	82	27	62	16	0[b]	4	0[b]	50	0[b,c]
12	13	100	34	100[c]	26	92[c]	5	80[c]	30	15	10	15[a]	11	0[b,c]

[a] >90% control.
[b] 100% control.
[c] Unreplicated.

Table 5. Effect of aerial applications of TH-6040 (Dimilin) and methoprene (Altosid SR-10) on natural populations of *Aedes taeniorhynchus* (mean of 2 replicates).

Dosage lb/acre (g/ha)		Pilot size acres (hectares)		% Eclosion: Pupae collected in plot	% Eclosion: Pupae caged within plot
			TH-6040		
0.025	(28)	4	(1.6)	8	0.5
		100[a]	(40)	0	0
0.05	(56)	4	(1.6)	0	0
			Methoprene		
0.025	(28)	30–100	(12–40)	0	0[b]
0.05	(56)	4	(1.6)	25	13
		15[a]	(7.6)	0	0
0.1	(112)	4	(1.6)	2	3
			Control		
0.0		4	(1.6)	98	80

[a] One replication.
[b] Limited eclosion occurred with pupae forming 5–6 days after treatment.

2 successive days so most of the larvae were washed away. Although the small 0.05 lb/acre plot was almost completely devoid of larvae or pupae after the rains, we were able to collect a standard sample of pupae (100) from the larger 0.025 lb/acre plot. Since no pupae in this sample eclosed, 100% control was achieved with the application of 0.025 lb/acre methoprene. The second test of methoprene was conducted on Galt Island, which is located west of Pine Island. Thirty acres (12 ha) were treated with 0.025 lb/acre of methoprene and the remaining 15 acres (6 ha) were treated with 0.05 lb/acre. A high canopy of 6- to 9-m trees uniformly covered the treatment plots, which contained *Ae. taeniorhynchus* larvae (10 to >250/dip). Pupae collected 48 and 72 hr after treatment failed to produce adults as did pupae collected the next 2 days. The 5th and 6th days posttreatment, limited numbers of exuviae were found in the plot treated with 0.025 lb/acre; but none were found in the plot treated with 0.05 lb/acre throughout the duration of the brood. Thus, the lower dosage rate was completely effective against pupae for the first 4 days, but residual activity was insufficient to completely prevent eclosion thereafter. Complete control was achieved with the higher dosage.

The aerial application of Dimilin was a single test conducted on the 100 acre plot at Wulfert's Point on Sanibel Island. Complete larval mortality was observed 24 hr after the application of 0.025 lb/acre. The rapid and complete larval kill suggested that lower dosages would also give complete control, because IGR activity is usually associated with concentrations lower than those that produce larval mortality.

The greater efficiency of both methoprene and Dimilin in the large-plot trials can be directly attributed to the improved coverage that results when larger areas are treated. The results of those tests made where the canopy was heavy are probably good indicators of the maximum dosages required to achieve adequate control of *Ae. taeniorhynchus*.

The decision was made to use aqueous applications of 5 gal/acre (7.7 liters/ha) in the aerial trials because we had observed the effects of normal wind and air currents on such operations on Sanibel Island in 1971 (unpublished data). In those trials ULV helicopter applications were subject to excessive drift; however, high volume application produced spray

placement and coverage that gave control of larvae.

At Galt Island, the methoprene might have had greater residual activity if more of the dose had actually reached the underlying water. We do not know what proportion of the 0.025 lb/acre applied actually filtered through the dense canopy, though enough did penetrate to give initial control. However, our small-plot studies (Table 4) suggest that 0.025 lb methoprene/acre have residual activity for more than 3 or 4 days. We therefore presume that the apparent reduction in residual activity is related to the difference between the rate of application and the rate of deposition on the target breeding water.

With Dimilin, which is a powerful larvicide, residual activity is not overly important; however, with methoprene it is because the compound is most effective during the 4th larval instar. In areas with the same type of breeding situation that was encountered among the mangroves, there are apt to be overlapping broods of *Ae. taeniorhynchus* larvae due to the continuous hatching that occurs when rainfall gradually expands breeding areas over a period of several days. Therefore, methods of increasing the efficiency of application of methoprene are needed to assure that a larger proportion of the active ingredient reaches the target and to reduce the total volume of active ingredient required to obtain control.

In our studies, a wide variety of compounds was effective against mosquito larvae in the laboratory, and several were effective in the small field plots. However, of those that were effective at practical levels (0.1 lb/acre or less) only methoprene and Dimilin were available for larger scale field trials. In the large-scale trials at 0.025 and 0.04 lb/acre, respectively, methoprene and Dimilin controlled *Culex* species. In flooded mangrove habitat, Dimilin provided complete control of *Ae. taeniorhynchus* larvae at 0.025 lb/acre and methoprene completely prevented adult eclosion at 0.05 lb/acre.

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