LABORATORY AND SMALL PLOT FIELD TESTS OF ALTOSID® AND DIMILIN® FOR THE CONTROL OF *AEDES TAENIORHYNCHUS* AND *CULEX NIGRIPALPUS* LARVAE

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ABSTRACT. Labortaory tests of Dimilin showed a decrease in susceptibility of fourth as compared to third instar larvae which was not evident in tests with Altosid. *Culex nigripalpus* was slightly more susceptible to Dimilin than was *Aedes taeniorhynchus*, but *A. taeniorhynchus* was considerably more suscetpible to Altosid than was *C. nigripalpus*.

Excellent results were obtained with experimental formulations of Altosid or Dimilin on sand or vermiculite against *A. taeniorhynchus* in simulated field tests conducted in the laboratory at 0.01 lb. A.I. per acre (0.0112 kg A.I. per ha.) and in small plot field tests at 0.02 lb. A.I. per acre (0.0224 kg A.I. per ha.). No difference in kill was noticed between second or third instar larvae in the small plot field tests.

The potential of juvenile hormone (JH) type compounds for use as insecticides was first evident in the now well known tests peformed with hormonally active extracts prepared from the male American silkworm moths *Hyalophora cecropia* by Williams in the early 1950's (Williams, 1956). Since that time, and particularly in the last several years, several JH analogs and other synthetic compounds have been developed which disrupt the normal development of insects.

Although several compounds are currently under development as mosquito larvicides; Altosid (methoprene) and Dimilin (TH-6040) definitely have shown promise as practical mosquito control agents. The tests of these compounds reported here deal not only with the standard available formulations but also with some experimental granular formulations which are believed necessary to penetrate the dense vegetation encountered in larval mosquito habitats in Florida when applied by aircraft.

MATERIALS AND METHODS. The dosage mortality tests were conducted in 600 ml. glass beakers containing a total of 250 ml. of water. Tap water was used in the tests with *Culex nigripalpus* Theobald and 25% sea water (9.0–11.5 ppt salt) was used in the tests with *Aedes taeniorhynchus* (Wiedemann). The beakers were placed in specially constructed water baths and held at 28° C ± 1°. In earlier tests, 10 third or early fourth instar larvae were placed in each beaker. In later tests with Dimilin against *A. taeniorhynchus* and Altosid against *C. nigripalpus*, 25 larvae per beaker were used, because it was felt that the higher numbers of larvae gave a more accurate percentage emergence. Both species used in the tests were from laboratory colonies.

Exposure to test compounds was continuous until pupation occurred. Pupae were removed daily, transferred to clean water and held to determine percent adult emergence. Treatment mortality, determined from adult emergence, was corrected by Abbott's formula. The LC_{50} and LC_{90} dosages were obtained from eye-fitted curves of the corrected percent mortality obtained at each dosage.

Each test consisted of 4 replications of 5 dosages and the check, and 2 to 5 tests were conducted with each compound and with each larval instar tested. A 25% wettable powder formulation of Dimilin and a 4 lb. per gal. emulsifiable concentrate formulation of Altosid were used to prepare the dilutions. Distilled water was used as a diluent for both compounds, and 1 ml. of the dilution was used per beaker with the check beakers receiving 1 ml. of distilled water only. All dilutions were

prepared immediately prior to each test.

Simulated field tests were conducted in 0.1 sq. m. circular metal pans 20 cm. deep lined with 6 mil polyethylene film. About 2 cm. of sandy soil was placed in the bottom of each pan and 4 liters of 25% sea water (7 to 9 ppt salt) were added to each pan. The pans were placed in a water bath, 2 pans per bath, and held at a temperature of 29° C ± 2° by means of aquarium heaters. The surface of the water in each bath was covered with pieces of styrofoam to limit heat dissipation from the water surface. Immediately prior to treatment, 200 third instar *A. taeniorhynchus* larvae were placed in each pan, and each test consisted of 2 replications of each treatment and the check. Larvae were fed daily a water mixture of powdered liver and brewer's yeast. After pupation began, pupae were removed daily, transferred to clean water and held at the same temperature in plastic cups, on top of which were mounted paper funnels and cylindrical screen cages to collect the emerging adults.

The formulations of the granular materials used in these tests are shown in Table 1. Except for the Altosid SR-10 on sand, the formulations are based on those developed for paris green by Rogers and Rathburn (1960). The oil and emulsifier mixture was added to the vermiculite or sand and blended evenly by stirring. Then the toxicant, or a blend of toxicant and calcium carbonate, in the case of the vermiculite formulations, was added and mixed thoroughly. The Altosid 20% premix was a dry formulation of Altosid and charcoal. The Altosid SR-10 on sand formulation was prepared by mixing the required amount of SR-10 to the sand and then adding the diatomite to form a dry formulation. All treatments were applied at a dosage rate of 0.01 lb. A.I. per acre (0.0112 kg. A.I. per ha.) and at a gross rate of 5 lb. per acre (5.6 kg. per ha.) and nothing was added to check pans. Although 0.02 lb. A.I. per acre is recommended for field applications, a dosage of only 0.01 lb. A.I. per acre was used in these tests because the dosage necessary to effect satisfactory control in the laboratory is generally considerably less than that required in field tests due to better coverage and more uniform testing conditions in the laboratory.

The small plot field tests were conducted in a specially constructed test area which consisted of 16 plots, each approximately 6 m. long, 3 m. wide and 30–45 cm. deep, arranged in a single line. A reservoir canal about 1 m. deep, which was surrounded by a dike 1 m. high, ran adjacent to the edge of all plots. Water from a nearby natural salt water lagoon was pumped into the canal, and each plot was flooded by means of gravity flow through a pipe placed through the reservoir dike above the water level in the plots. The

Table 1. Granular formulations of Altosid and Dimilin on sand and vermiculite used in the laboratory and field tests.[1]

Insect growth regulator	Formulation	Percent by weight
Altosid	20% premix	1.0
	calcium carbonate	34.0
	Gulf 562 oil+3% Triton N101	42.0
	20–40 mesh vermiculite	23.0
Dimilin	25% wettable powder	0.8
	calcium carbonate	34.2
	Gulf 562 oil+3% Triton N101	42.0
	20–40 mesh vermiculite	23.0
Altosid	20% premix	1.0
	Gulf 562 oil+3% Triton N101	1.0
	20–45 mesh sand	98.0
Dimilin	25% wettable powder	0.8
	Gulf 562 oil+3% Triton N101	0.2
	20–45 mesh sand	99.0
Altosid	SR-10 (10% methoprene)	2.0
	diatomite	5.0
	25–40 mesh sand	93.0

[1] Formulations shown are for 0.02 lb. A.I. (0.0224 kg./ha.) per acre at a gross rate of 10 lb. per acre (11.2 kg./ha.) or 0.01 A.I. per acre (0.0112 kg./ha.) at a gross rate of 5 lb per acre (5.6 kg./ha.). The percent of toxicant was doubled and the calcium carbonate decreased by the same amount when used at 0.02 lb. A.I. (0.0224 kg./ha.) per acre at a gross rate of 5 lb. per acre (5.6 kg./ha.).

pipes in all plots were placed at the same level so that a float switch mounted in the first plot maintained the same water level in each plot.

The salinity of the water in Tests 1 and 2 averaged 20.7 and 26.4 ppt salt respectively. In Test 3, the salinity averaged only 2.3 ppt because the plots were flooded by rain and not by water from the salt water lagoon. The water depth in all plots averaged 10–20 cm., and the daytime temperature of the water usually ranged between 30 and 35° C., but occasionally reached 40° C.

In Tests 1 and 2, *A. taeniorhynchus* larvae from the laboratory colony were reared in the insectary in salt water taken from the canal, and approximately 2,000 to 3,000 larvae were placed in each plot about 4 hrs. before treatment. In Test 3 a natural population of *A. taeniorhynchus* larvae resulting from flooding by heavy rains was used. At the time of treatment the larvae in Test 1 were in the third instar, in Test 2 third instar with some fourth instar, and in Test 3 mostly second instar. All treatments and the checks were replicated twice except that 1 check plot was used in Test 3. The liquid formulations were applied manually by use of a clothes sprinkler and the granular formulations by means of a glass jar with 14 by 18 mesh screen top. The water surface was measured prior to each treatment and the correct amount of formulation was weighed for each plot. The formulations of the granular materials used in these tests are shown in Table 1. The granular formulations used in the check plots were the same as used in the treated plots but without the toxicant. All treatments were applied at a rate of 0.02 lb. A.I. per acre (0.0244 kg. per ha.) as recommended by the manufacturers at a gross rate of either 5 or 10 lb. per acre (5.6 or 11.2 kg. per ha.).

Ten dips per plot were taken pre- and posttreatment, 5 from each side of the plot and, when possible, only where larvae were observed. Pupal collections for observation of adult emergence were made each day starting when the first pupae appeared in appreciable numbers and were continued until pupae were no longer present. A minimum of 50 pupae per plot per day were collected when possible; however, in most treated plots all the live pupae observed were sampled since this number was usually less than 50 per plot.

RESULTS. The results of the laboratory dosage-mortality tests are shown in Table 2. There appeared to be a decrease in susceptibility of later instar larvae to Dimilin which was not evident with Altosid. *C. nigripalpus* was more susceptible to Dimilin than *A. taeniorhynchus,* but *A. taeniorhynchus* was considerably more susceptible to Altosid than *C. nigripalpus.*

Results of the simulated field tests are shown in Table 3. Excellent results were obtained with both the sand and vermiculite formulations of both Altosid and Dimi-

Table 2. Results of laboratory dosage-mortality tests of Dimilin and Altosid against *Aedes taeniorhynchus* and *Culex nigripalpus* larvae.

Insect growth regulator	Mosquito species	Instar	Total no. reps	Lethal concentration in μg/ml.	
				LC_{50}	LC_{90}
Dimilin	*Aedes taeniorhynchus*	3	20	0.00033	0.00069
		4	20	0.00093	0.00205
	Culex nigripalpus	3	8	...	0.00016
		4	8	0.00028	0.00080
Altosid	*Aedes taeniorhynchus*	3	8	0.00019	0.01550
		4	16	0.00023	0.01450
	Culex nigripalpus	3	16	0.00260	0.35000

Table 3. Results of laboratory tests of various formulations of Dimilin and Altosid applied at 0.1 lb. A.I. (0.0112 kg./ha.) per acre rate against *Aedes taeniorhynchus* larvae.

Insect growth regulator	Formulation [1]	Percent mortality		Percent adult emergence [4]
		larvae [2]	pupae [3]	
Dimilin	25% w.p. on sand	100	..	0
Dimilin	25% w.p. on vermiculite	100	..	0
Check	untreated	8.5	9.3	83.0
Altosid	20% premix on sand	25.5	100	0
Altosid	20% premix on vermiculite	24.0	100	0
Check	untreated	20.0	25.0	60.0
Altosid	SR-10 in water	21.3	100	0
Altosid	SR-10 on sand	8.8	100	0
Check	untreated	7.5	13.0	80.5

[1] SR-10 applied at 1.5 fl. oz. per acre rate (110 ml./ha.) diluted in water, all others at 5 lbs. per acre gross rate (5.6 kg./ha.). w.p. = wettable powder.
[2] Based on initial number of larvae and number of pupae collected.
[3] Based on number of pupae collected and number of adults produced.
[4] Based on initial number of larvae and number of adults produced.

lin. Even at the low dosage rate of 0.01 lb. A.I. per acre (0.0112 kg./ha.), there was no emergence.

Results of the small plot field tests are shown in Tables 4 and 5. Given in Table 4 are the actual dip counts of larvae and pupae. It is evident that none or only very few pupae were formed in the plots treated with Dimilin while the bulk of the mortality by Altosid was in the pupal stage. Although taking approximately one day longer to effect final kill, Altosid in Test 3 against second instar larvae was as effective as in Test 1 and 2 with third instar larvae. It is evident from the data in Table 4 that while dead pupae were much in evidence, dead larvae were not as readily observed. This was probably due to the fact that the dead larvae rapidly sink while the pupae tend to float for a longer time before they sink. Many dead pupae were also noticed on the bottom as well as on the water surface in Test 3 but probably were more noticeable because of the higher initial population and the clarity of the water in this test.

Many emerging adults were noticed on the water surface in the check plots in Test 1 at 72 and 96 hours, in Test 3 at 96 and 120 hours, and many cast pupal skins, indicating a previous emergence, were noted on the water surface in the check plots in Test 2 at 96 hours. No cast pupal skins or emerging adults were noticed in any of the treated plots in any test.

Although no attempt was made to assess the effect of the treatments on non-target organisms in these tests, several live dytiscid larvae and many corixids (up to several hundred per dip) were noted in both treated and check plots during the dipping in Tests 2 and 3.

The emergence of adults from the *A. taeniorhynchus* pupae collected from the field plots is shown in Table 5. No adults emerged from the pupae collected from any of the Altosid treated plots except for 6 that emerged from the first sampling of Plot 1 in Test 2 which was treated at the 5 lb. per acre rate (5.6 kg./ha.). Very few live pupae were observed in the Altosid treated plots in Tests 1 and 2. In fact, the figures shown in Table 5 represent all the live pupae that could be found in the Altosid treated plots. In these tests, no live pupae were observed in any plot treated with Dimilin at the time the pupal collections were made; however, as shown in Table 4, 1 isolated pupa was observed at 24 hours in 1 Dimilin plot in Test 2.

DISCUSSION. In comparing the results of dosage-mortality data obtained in these

Table 4. **Results of small plot field tests of various formulations of Altosid and Dimilin against *Aedes taeniorhynchus* applied at 0.02 lb. A.I. per acre (0.0224 kg./ha.) rate.**

Test No.	Insect growth regulator	Formulation	Gross rate per acre	Number larvae (alive and dead) and pupae (alive and dead) per 20 dips[1]																	
				Pre	24 hr.				48 hr.				72 hr.				96 hr.				120 hr.			
				larvae	larvae		pupae		larvae		pupae		larvae		pupae		larvae		pupae		larvae		pupae	
				al	al	dd	al	dd	al	dd	al	dd	al	dd	al	dd	al	dd	al	dd	al	dd	al	dd
1	Altosid	Spray[2]	5 gals.	155	48	0	0	0	95	0	1	4	27	0	8	15	0	0	3	37	0	0	0	0
	Dimilin	Spray[3]	5 gals.	248	107	0	0	0	145	0	0	0	13	0	8	1	3	3	0	0	0	0	0	0
	Check	....	....	98	62	0	0	0	122	0	7	0	59	0	37	0	5	0	44	0	0	0	0	0
2	Altosid	granular	5 lbs.	187	259	0	12	0	251	0	0	8	55	4	13	19	13	0	28	51	1	0	1	45
			10 lbs.	197	152	1	0	0	186	1	0	0	121	0	33	21	52	3	45	23	1	1	0	29
	Dimilin	granular	5 lbs.	141	220	0	0	0	102	3	0	0	29	11	0	0	0	8	0	0	0	3	0	0
			10 lbs.	129	172	1	1	0	114	4	0	0	24	10	0	2	1	4	0	4	0	0	0	1
	Check	granular	10 lbs.	181	154	0	7	0	81	0	9	0	32	0	63	0	6	0	48	0	3	0	3	0
3	Altosid	granular	10 lbs.	329	..	..	..	..	262	0	0	0	286	0	17	4	212	0	217	0	9	0	65	167
	Check	granular	10 lbs.	219	..	..	..	..	139	0	0	0	187	0	41	0	165	0	42	0	15	0	24	0

[1] No larvae or pupae present at 144 hrs. in Test 2 and 3; al = alive, dd = dead.
[2] SR-10 diluted in water.
[3] 25% wettable powder diluted in water.

Table 5. Emergence of *Aedes taeniorhynchus* adults from pupae collected in small field plots treated with Altosid and Dimilin at 0.02 lb. A.I. per acre rate (0.0224 kg./ha.).

Test No.	Insect growth regulator	Formulation	Gross rate per acre	Number pupae collected	Number adults produced	Percent emergence
1	Altosid	Spray [1]	5 gals.	42	0	0
	Dimilin	Spray [2]	5 gals.	0	0	0
	Check	untreated	....	183	165	90.2
2	Altosid	granular	5 lbs.	78	6	7.7
			10 lbs.	83	0	0
	Dimilin	granular	5 lbs.	0	0	0
			10 lbs.	0	0	0
	Check	granular	10 lbs.	161	138	85.7
3	Altosid	granular	10 lbs.	379	0	0
	Check	granular	10 lbs.	301	254	84.4

[1] SR-10 diluted in water.
[2] 25% wettable powder diluted in water.

tests with those of other investigators, there appears to be a wide variation in the LC_{50} and LC_{90} with both Altosid and Dimilin against the same species (Schaefer and Wilder 1972 and 1973; Jakob 1972; Henrick et al. 1973; Brown and Brown 1974; and Hsieh and Steelman, 1974; Thompson-Hayward Technical Bulletin, 1974). Much of this variation is undoubtedly due to differences in testing procedures such as the use of different strains of mosquitoes, larval instars, rearing temperatures, water type, food, light, formulations, etc. Cross resistance to other insecticides demonstrated in flies by Cerf and Georghiou (1972 and 1974) may also be an important factor in the variations obtained by different investigators. Schaefer and Wilder (1972) also experienced variations between individual tests on the same strains of fourth instar larvae and attributed the variation to larval age within a given instar at treatment time.

Contrary to results of Schaefer and Wilder (1972), who reported Altosid to be less effective on third instar than on fourth instar *C. pipiens quinquefasciatus* and *A. nigromaculis* larvae, little difference was noted in these tests between third and fourth instar *A. taeniorhynchus*. With Dimilin, however, there was a reduction in susceptibility noted in these tests with fourth instar *A. taeniorhynchus* and *C. nigripalpus* larvae as compared to third instar larvae. This is also reported in the Thompson-Hayward Technical Bulletin (1974) for *A. vexans, C. pipiens pipiens* and *C. pipiens quinquefasciatus*.

In field tests of the emulsifiable concentrate formulation of Altosid, Schaefer and Wilder (1973) obtained good results only on fourth instar larvae; however, in tests with the 10% flowable liquid, a slow release formulation of Altosid, they obtained good results against second, third and fourth instar larvae with dosages as low as 0.025 lb. A.I./acre in aerial applications and as low as 0.01 lb. A.I./acre in hand applications. No differences in kill were noted between second and third instar larvae in the small plot field tests reported here, which were conducted with either Altosid or Dimilin at a dosage of 0.02 lb. A.I./acre (0.0224 kg./ha.).

Although water formulations of both Altosid and Dimilin gave excellent results in the field trials with hand applications, future tests in Florida will be directed toward developing granular formulations of both IGR compounds for aerial application over areas of dense vegetation, such as mangrove swamps.

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