METHOPRENE OR ALTOSID FOR THE CONTROL OF *AEDES DETRITUS* AND ITS EFFECTS ON SOME NON-TARGETS

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ABSTRACT. The insect growth regulator Altosid® SR–10 was evaluated against larvae of the floodwater mosquito *Aedes (Ochlerotatus) detritus* Haliday, 1833 in a salt marsh in the Province of Grosseto, Italy.

In laboratory tests, the LC_{50} and LC_{90} values for Altosid SR–10 were 0.0009 and 0.0085 ppm respectively.

Altosid SR–10 when applied at 30 g AI/hectare (0.026 lb AI/acre) to natural salt marsh pools yielded complete inhibition of adult emergence up to 4 days, while at 60 g AI/hectare (0.052 lb AI/acre) inhibition of emergence was complete for the duration of the test (8 days).

At both rates used, diving beetles showed a tolerance and copepod population showed non-marked short-term reduction.

The SR–10 formulation applied at the rate of 40 g AI / hectare (0.035 lb AI / acre) in a 4,550 m^2 salt marsh provided complete control of *A. detritus* for 4 days after treatment; adult emergence inhibition did not drop below 50% until the 16th day.

The residual properties of this microencapsulated formulation, offering a period of emergence inhibition of about two weeks, as found in these studies, are excellent for control of floodwater mosquitoes.

INTRODUCTION

One of the most common mosquitoes in the Province of Grosseto (Italy) is *Aedes (Ochlerotatus) detritus* Haliday, 1833 the larvae of which are found in brackish waters. The adults of this species appear in significant numbers from the beginning of autumn to the beginning of summer, and 1st stage larvae appear soon after rain and/or tides (Maroli et al. 1973). During the breeding season several waves of emergence take place. The adults are highly active during the early and late hours of the day, attacking man and animals in great numbers. So far this species is considered to be annoying and a cause of discomfort, but it has not been demonstrated to be a vector of viruses affecting man.

The use of insect growth regulators (IGR's) has shown good potential for the control of flood water and other mosquitoes (Lewallen and Ramke 1974, Mulla and Darwazeh 1975, Schaefer and Wilder 1973, Schaefer et al. 1974, Steelman et al. 1975).

The aim of the present work was to determine the efficacy of a slow release IGR against *Ae. detritus* larvae in the laboratory and in a marshy area of the Tirrenean coast 15 km south of Grosseto, and to evaluate its effects on some non-target aquatic organisms.

MATERIALS AND METHODS

Methoprene or Altosid® SR–10/Isopropyl (E,E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate/10% microencapsulated slow release (Zoecon Corp.) was employed in these studies.

A—LABORATORY. *Ae. detritus* larvae were brought to the laboratory from the salt marsh. To determine the LC_{50} and LC_{90} values, the Altosid SR–10 formulation was serially diluted with distilled water and tested against 4th-stage larvae. Twenty larvae were placed in 200 ml of field-collected water in 350 ml capacity glass beakers, and fed only once, to avoid massive microbial growth, with wheat bran mixed with

fish food. Tests were carried out at 21°C and a photoperiod of 12 hours light : 12 hours dark. Each concentration was run in duplicate and all tests were replicated 3 times. For the evaluation of adult emergence inhibition, procedures described by Mulla et al. (1974) were utilized. To allow normal adults to fly away, test units were left uncovered. Mortality was assessed every 2 days, and dead or moribund mosquitoes were removed from the beakers. Adult emergence was determined by counting fully separated exuviae, dead larvae and pupae during the course of the experiments. Overall activity was assessed as percent inhibition of emergence corrected for checks by the formula $100 - \frac{T}{C} \times 100$, where T=percent emergence in treated and C = percent emergence in checks. The corrected percent values were plotted on log probit scale against concentration.

B—FIELD. Field tests were carried out in a marsh separated from the sea by a 15 m wide sand dune, subject to the action of the sea which, during winter in particular, sometimes flows over the sand dune. The vegetation of the breeding places consisted mainly of *Juncus acutus* and *J. maritimus*.

Test 1. Because the marsh had not been completely flooded for evaluation since April 1975, 5 pools of uniform size were isolated by means of zinc-plated sheet metal. The surface of each pool was 12 m^2 and the water depth ca. 50 cm (45–55 cm). Water temperature averaged 19°C (18–21°C). Two pools were treated with Altosid SR–10 at a concentration of 30 g AI/hectare (0.026 lb AI/acre), 2 at a concentration of 60 g AI/hectare (0.052 lb AI/acre) and one was left untreated as a check. It was calculated that the initial concentrations had been diluted in the pools to about 0.06 and 0.12 ppm respectively. The required amount of material was mixed with 500 ml of water and applied with an all-purpose sprayer (1.5 liter capacity). All stages of larvae were present with 3rd-stage larvae predominant at time of treatment.

Procedures for determining effects of the material on mosquito larvae and nontarget organisms described by Mulla et al. (1974, 1975) were employed. In brief, to determine the percentage of emergence inhibition (%EI) twenty 4th instar *A.detritus* were placed, 24 hr after treatment, in each of 2 floating isolation units per pond. The unit consists of a 1-qt polystyrene cup which rests inside a styrofoam ring float. Four holes are spaced and cut around the periphery of the cup—1.5 cm from its base and a 100-mesh brass strainer cloth is soldered to cover the holes. For collecting adult mosquitoes, a ventilated collection chamber was attached to the top of the unit. At intervals after treatment, 2 floating units/pond were supplied with 4th-instars from the same ponds. These isolated individuals were observed until emergence. Mortality and survival of each stage and adult emergence were assessed every other day. The percent inhibition of emergence based on the number of larvae was corrected with the aforementioned formula.

Nontarget organisms were sampled by a dipper. Five dips per plot were taken from fixed sampling sites before treatment and 2,4,8 and 14 days after treatment. The 5 samples were composited and concentrated in a measuring cup provided with 150-mesh stainless steel cloth affixed to a cutout section 1.5 cm above the bottom. The concentrated sample was transferred to plastic vials, preserved by addition of 95% ethyl alcohol and examined in the laboratory under a stereoscopic microscope.

Test 2. During October 1975 the marsh was completely flooded. The water depth was about 30 cm (5–40 cm). The temperature of the water dur-

ing the day averaged 23.1°C (16.0–25.5°C) and chlorine ion concentration was 14 g/liter (12–16 g/liter). A surface of 4,550 m^2 (1.123 acres) was then treated with Altosid SR–10 at a rate of 40 g AI/ha (0.035 lb AI/acre). The material was applied using a pressurized hand sprayer of 12 liter capacity. An isolated plot equal in size and presenting similar characteristics was chosen as check at a distance of 40 m from the treated area.

Larval density and stage composition was determined 24 hr before treatment by taking 50 dips. The average number of larvae and pupae/dip was 94.7 (2nd stage 12.4%, 3rd stage 26.6%, 4th stage 41.8% and pupae 19.2%) and 37.5 in the check area (2nd stage 1.4%, 3rd stage 15.0%, 4th stage 47.0% and pupae 36.6%).

Because of dense vegetation, floating units were difficult to place and, therefore, not used. To evaluate emergence inhibition, 4th-stage larvae and pupae were collected from the treated salt marsh at fixed intervals, for several days after treatment. Three hundred 4th-stage larvae, randomly collected from the treated salt marsh, were put in 4 liter glass beakers (50 in each) each containing 2 liters of treated water. One hundred pupae were also collected at intervals after treatment and distributed in 2 beakers containing 2 liters of treated water. The pupae were collected 8 days after treatment, i.e. when old pupae would all have disappeared. The beakers covered with gauze netting were left outdoors in a sheltered place. Mortality of each stage and adult emergence were assessed every 2 or 3 days.

For bioassay of treated field water, 100 4th-stage larvae were randomly collected from an untreated portion of the marsh next to the check area and placed in 2 beakers (4 liter capacity) each containing 2 liters of water. Water was collected at 5 cm below the surface in the middle of each area. The water was then bioassayed on the spot against field-collected larvae.

RESULTS AND DISCUSSION

LABORATORY. Altosid SR-10 proved to be highly effective against *Ae. detritus* showing LC_{50} and LC_{90} values of 0.0009 and 0.0085 ppm. These preliminary results were encouraging, and warranted field evaluation.

Test 1. Altosid SR–10 in natural salt marsh ponds at 30 g AI/ha yielded complete inhibition of adult emergence of *A. detritus* up to 4 days after treatment. Emergence inhibition decreased to 90% at 8 days after treatment. In the same test, at the rate of 60 g AI/hectare inhibition of emergence was complete (Table 1) for 8 days.

Due to lack of larvae, it was not possible to make observations beyond 8 days. It should be noted that at the highest rate mortality occurred mainly in the larval stage. This could be explained by the high susceptibility of the species to the IGR tested.

NONTARGET ORGANISMS. In Table 2 are shown the results of the effects of Altosid SR–10 on diving beetles (Coleoptera) and on zooplankton (Copepoda) by evaluating pre- and post-treatment population samples. The Coleoptera were represented by 4 dytiscid species: *Potamonectes cerisyi* Aubé, *Coelambus parallelogrammus* Ahr., *Hydroporus limbatus* Aubé and *Hydaticus leander* Rossi. The Copepoda were represented by Cyclopidae, *Cyclops* spp.

It does not appear that Altosid SR–10 at either rate causes a reduction in diving beetle populations. The same can be said for the copepods whose population fluctuation trends during the 2-week test period appear to be comparable to those of the check, at

Table 1. Mortality and emergence inhibition of *Aedes detritus* from isolated 4th stage larvae in natural pools treated with Altosid SR-10

	Post-treat (days)	Avg. (%) cumulative mortality at indicated rates (g AI/hectare) [a]							
		30				60			
		L	P	A	(%) EI [b]	L	P	A	(%) EI
Altosid SR-10	2	45	54	1	100	60	40	0	100
	4	22	78	0	100	62	38	0	100
	8	30	60	0	90	45	55	0	100
	14 [c]	..	..	..	..	..	..	..	..
Check	2	10	5	0	15	..	..	..	..
	4	5	3	0	8	..	..	..	..
	8	7	5	0	12	..	..	..	..
	14 [c]	..	..	..	..	..	..	..	..

[a] Two isolation units containing 20 4th-stage larvae/unit were introduced in each pool at indicated days after treatment.
[b] L=larvae; P=pupae; A=adults; EI=emergence inhibition.
[c] Not enough 4th-stage larvae present in the pools for isolation units.

both concentrations. Miura and Takahashi (1974) reported that in field tests copepods showed some susceptibility at the dose of 0.025 lb AI/acre, but they concluded that the rates used for mosquito control are probably safe to use in irrigated pastures. Norland and Mulla (1975) reported that repeated treatments of Altosid EC_4 to experimental ponds at the rate of 0.1 ppm (0.27 lb/acre) reduced abundance of several arthropod prey and predator species. A major predator, the larval dytiscid beetle, *Laccophilus* sp., was eliminated from the treated ponds. Steelman et al. (1975) found a reduction in the *Tropisternus* spp. adult population caused by 4 IGRs including Altosid.

Test 2. Table 3 shows the results obtained from hand spray application of Altosid SR–10 at the rate of 40 g AI/ha (0.035 lb AI/acre) for the control of *A. detritus*. In this treatment inhibition of adult emergence from field-collected larvae was almost complete for 4 days after treatment and did not drop below 50% until the 16th day. Inhibition of adult emergence from field-collected pupae was 100% 8 days after treatment, 86% and 40% 16 and 22 days after treatment respectively.

These results are in good agreement with those of Arevad (1973) in Den-

Table 2. Effects on some nontarget organisms in pools treated with Altosid SR-10.

Rate (gAI/hectare)	Avg. no. of specimens/5 dips pre- and post-treat (days)									
	Coleoptera [a]					Copepoda [b]				
	Pre	2	4	8	14	Pre	2	4	8	14
30	4	3	4	7	8	6	3	16	42	11
60	6	2	5	2	10	4	4	10	13	7
Check	3	8	7	11	8	3	3	18	26	24

[a] Dytiscidae, *Potamonectes cerisyi* Aubé, *Coelambus parallelogrammus* Ahr., *Hydroporus limbatus* Aubé and *Hydaticus leander* Rossi.
[b] Cyclopidae, *Cyclops* spp.

Table 3. Field evaluation of Altosid SR-10 applied at the rate of 40 g AI/hectare on a 0.455 hectare salt marsh against *Aedes detritus* larvae.

	Avg. % cumulative mortality and inhibition of emergence (%)									
	Larval isolates				pupal isolates			Bioassay of field collected water		
Post-treat (days)	L	P	A	(%) EI	P	A	(%) EI	L	P	A	(%) EI
2	20	75	1	96	..	..	..	..	..	..	..
4	32	67	1	100	..	..	..	..	..	..	..
8	17	66	3	86	100	..	100	7	15	0	22
16	8	50	0	58	86	0	86	6	8	1	15
22[a]	8	22	0	30	36	4	40[b]	..	..	..	..
Check											
2	8	4	0	12	..	..	..	..	..	..	..
4	7	7	0	14	..	..	..	..	..	..	..
8	4	12	2	18	6	1	7	4	8	0	12
16	6	10	0	16	6	2	8	5	3	0	8
22[c]	..	..	..	..	..	..	..	..	..	..	..

L=larvae; P=pupae; A=adults; EI=emergence inhibition.
[a] Only 115 4th-stage larvae were found.
[b] Only 46 pupae were found.
[c] Insufficient number of larvae and pupae found.

mark who found a suppression of mosquito emergence for a period of 15–20 days, by using Altosid SR–10 at the same rate/ha against a natural population of salt marsh mosquitoes among which were *Ae. detritus*.

The bioassay results on water taken from the field at the 8th and 16th days after treatment (Table 3) indicate that little or no residual activity was detected. This could be explained with an uneven distribution of the compound through the whole body of water, the higher concentration occurring in the upper layers or on the lee side of the area (Schaefer and Dupras 1973).

In conclusion, emergence inhibition for more than 2 weeks, as we found, can only be attributed to the direct residual activity of this slow release formulation. It is thus concluded that the slow release (SR–10) formulation of Altosid offers a good tool for the control of floodwater mosquitoes.

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References

Arevad, K. 1973. Juvenile hormone mimic against mosquito larvae. A small-scale field test with a new formulation. Danish Pest Inf. Lab. Ann. Rep. pp. 47–49.

Lewallen, L. L. and Ramke, D. A. 1974. Extensive application of Altosid as a mosquito larvicide. Proc. Calif. Mosq. Cont. Assoc. 42:119.

Maroli, M., Majori, G., Turillazzi, P., Bettini, S. and Pierdominici, G.1973. Osservazioni sulla biologia di *Aedes detritus* in alcuni focolai larvali della Maremma toscana. Riv. Parassit. 34:219–232.

Miura, T. and Takahashi, R. M. 1974. Insect developmental inhibitors. Effects of candidate mosquito control agents on non-target aquatic organisms. Envir. Entomol. 3:631–636.

Mulla, M. S. and Darwazeh, H. A. 1975. Evaluation of insect growth regulators against

Psorophora confinnis (L-A) in southern California. Mosquito News 35:281–285.

Mulla, M. S., Darwazeh, H. A. and Norland, R. L. 1974. Insect growth regulators: evaluation procedures and activity against mosquitoes. J. Econ. Entomol. 67:329–332.

Mulla, M. S., Majori, G. and Darwazeh, H. A. 1975. Effects of the insect growth regulator Dimilin or TH-6040 on mosquitoes and some nontarget organisms. Mosquito News 35:211–216.

Norland, R. L. and Mulla, M. S. 1975. Impact of Altosid on selected members of an aquatic ecosystem. Envir. Entomol. 4:145–152.

Schaefer, C. H. and Dupras, E. F., Jr. 1973. Insect developmental inhibitors. 4. Persistence of ZR-515 in water. J. Econ. Entomol. 66:923–926.

Schaefer, C. H. and Wilder, W. H. 1973. Insect developmental inhibitors. 2. Effects on target mosquito species. J. Econ. Entomol. 66:913–916.

Schaefer, C. H., Wilder, W. H., Mulligan, F. S. III and Dupras, E. F., Jr. 1974. Insect developmental inhibitors: effects of Altosid, TH-6040 and H 24108 against mosquitoes (Diptera: Culicidae). Proc. Calif. Mosquito Cont. Assoc. 42:137–139.

Steelman, C. D., Farlow, J. E., Breaud, T. P. and Schilling, P. E. 1975. Effects of growth regulators on *Psorophora columbiae* (Dyar and Knab) and nontarget aquatic insect species in rice fields. Mosquito News 35:67–76.

FIELD SURVIVAL AND OVIPOSITIONAL CHARACTERISTICS OF *AEDES AEGYPTI* AND THEIR RELATION TO POPULATION DYNAMICS AND CONTROL

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ABSTRACT. Approximately 34,000 adult *Aedes aegypti* (L.) were released on Seahorse Key, an island off the Gulf coast of Florida. Ovitraps were used to collect eggs over a 21-day period after the release to determine oviposition cycles, and adult females were caught to assess the number of eggs in a single oviposition. Oviposition began 5 days after release, and peaks occurred at 4-day intervals. The average number of eggs for a single oviposition was 93±6. A mean daily adult survival rate of 0.82 was calculated by linear regression of egg density on time (days) after release. Larval survival was strongly density-dependent in tests conducted in cages under ambient conditions. The adult survival and fecundity data were used to calculate rates of immature survival corresponding to assumed rates of population growth.

Our interest in genetic methods for controlling *Aedes aegypti* (L.) has led to experimental evaluations of the competitiveness of genetically-altered males in limited field tests (Seawright et al., 1975, 1976). During the course of these tests we obtained information concerning ovipositional cycles and fecundity of adult females. We also studied the survival of larvae at various densities. These data are presented here to give a partial view of the dynamics of *A. aegypti* in north Florida and to indicate the relevance of genetic control methodology to this species under these climatic conditions.

METHODS AND MATERIALS

ADULT STUDY. All larval rearing for the adult releases was done in an outdoor cage using water from a subterranean well. Groups of 1400 first stage