SMALL PLOT EVALUATION OF A SUSTAINED-RELEASE SAND GRANULE FORMULATION OF METHOPRENE (SAN 810 I 1.3 GR) FOR CONTROL OF *AEDES TAENIORHYNCHUS*[1]

DANIEL L. KLINE

U.S. Department of Agriculture, Agricultural Research Service, Medical and Veterinary Entomology Research Laboratory, P.O. Box 14565, Gainesville, FL 32604

ABSTRACT. A sand granule formulation of methoprene (SAN 810 I 1.3 GR) was tested in outdoor intermittently flooded pools as a pre- and postflood treatment for the control of *Aedes taeniorhynchus*. In field test 1, pre- and postflood treatments were equally effective. Inhibition of emergence in mosquitoes exceeded 90% for one and 3 flood/dry cycles when SAN 810 I 1.3 GR was applied at rates of 2.8 and 5.6 kg/ha, respectively. In field test 2, >90% inhibition of emergence was achieved only when SAN 810 I 1.3 GR was applied postflood at 5.6 kg/ha.

INTRODUCTION

Aedes taeniorhynchus (Wied.) is a major mosquito pest in coastal areas of North America. Female mosquitoes lay their eggs on the soil above receding waterlines in saltmarsh and mangrove habitats. When inundated, eggs hatch and produce large broods of larvae. To prevent emergence of adult mosquitoes, these breeding sites must be treated with larvicides or insect growth regulators (IGRs) each time they are flooded. Inclement weather following flooding can delay or prevent treatment of breeding sites, thus allowing the immature mosquitoes to pupate and emerge. These difficulties could be overcome by using a mosquito control agent that could be applied prior to breeding site inundation and that demonstrates residual toxicity for several consecutive floodings.

A study was conducted with a sustained-release formulation of methoprene (SAN 810 I 1.3 GR) to determine if it met these criteria. Specific objectives were: 1) to determine the number of flood/dry cycles (of 2 wk duration) through which this formulation will control saltmarsh mosquitoes, and 2) to determine if the rate and/or timing of application (i.e., pre- versus postflooding) affected the duration of control.

MATERIALS AND METHODS

Studies were conducted in outdoor concrete test pools specially constructed so that they would have uniform size and volume (Focks and Bailey 1983). The pools were lined with St. Augustine grass sod to simulate actual floodwater mosquito larval habitats.

Two field tests were conducted (May 1–July 4 and July 31–September 28) in 1990. Each test consisted of 4–5 flood/dry cycles. Each flood/dry cycle lasted 14 days, i.e., pools remained flooded for 7 days then were drained and kept dry for 7 days. Each cycle was initiated by simultaneously flooding all pools. Three days after flooding, ca. 1,000 late 3rd to early 4th instar *Ae. taeniorhynchus* larvae, obtained from an insecticide susceptible colony maintained at the USDA Medical and Veterinary Entomology Research Laboratory, were introduced into each pool. After an additional 4 days, 100 pupae were collected from each pool and placed in water from the respective plot in plastic-lined 0.5-liter (pint) ice cream containers. These containers were stored at room temperature and observed daily for adult emergence. When adult emergence was complete, dead pupae (DP), dead adults (DA) on the surface of the water, and live adults (AA) in each container were counted and recorded. From these data the % Emergence Inhibition (% E. I. = % control) for each plot was calculated using the formula:

$$\%\ \text{E. I.} = \frac{(\text{DP} + \text{DA})}{\text{DP} + \text{DA} + \text{AA})} \times 100.$$

Seven days after flooding, the pools were drained and kept dry for 7 days except for natural rainfall. The period between flooding and the subsequent reflooding of a pool was considered a flood/dry cycle. In each test, the flood/dry cycle was repeated at least one time *after* percent E. I. in all treated pools fell below 70%. Test pools were kept dry, except for natural rainfall, for 16 days between the 2 tests.

In each test, treatments were assigned pools on the basis of a completely randomized design. Each treatment was replicated 3 times and comprised one of 2 rates of application (2.8 kg/ha [2.5 lbs/acre] or 5.6 kg/ha [5 lbs/acre]) of a 1.3% sustained-release formulation of methoprene

[1] Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the United States Department of Agriculture.

Table 1. Small plot evaluation of SAN 810 I 1.3 GR (sustained-release formulation of 1.3% methoprene) for the control of *Aedes taeniorhynchus* in intermittently flooded artificial habitats (pools)

	Mean[a] percent emergency inhibition				
	2.8 kg/ha		5.6 kg/ha		
Flood/dry cycle no.	Preflood Mean ± SE	Postflood Mean ± SE	Preflood Mean ± SE	Postflood Mean ± SE	Check Mean ± SE
Field test 1 (May 11–July 6, 1990)					
1	93.0 ± 2.5 A	100.0 ± 0.0 A	97.0 ± 3.0 A	99.7 ± 0.3 A	12.0 ± 2.5 B
2	50.7 ± 21.1 A	58.0 ± 23.1 A	92.3 ± 4.1 A	87.3 ± 9.7 A	1.0 ± 0.6 B
3	58.3 ± 8.9 B	62.0 ± 9.2 B	93.7 ± 3.5 A	93.3 ± 5.7 A	3.7 ± 1.8 C
4	2.3 ± 1.5 B	3.7 ± 2.3 B	4.3 ± 0.7 B	10.3 ± 2.2 A	1.3 ± 0.7 B
5	5.0 ± 0.6 A	4.3 ± 1.9 A	4.7 ± 0.3 A	4.0 ± 1.2 A	4.0 ± 1.2 A
Field test 2 (August 10–September 21, 1990)					
1	57.3 ± 6.6 B	63.7 ± 9.8 B	84.3 ± 3.4 A	97.0 ± 0.6 A	11.7 ± 4.8 C
2	56.3 ± 2.7 B	76.7 ± 11.3 A	79.0 ± 4.5 A	92.7 ± 2.7 A	11.3 ± 2.9 C
3	8.0 ± 4.6 B	18.7 ± 5.6 B	11.7 ± 3.7 B	40.0 ± 10.9 A	6.3 ± 1.2 B
4	8.7 ± 1.8 C	26.0 ± 3.7 B	11.3 ± 2.9 C	38.7 ± 1.2 A	4.7 ± 3.7 C

[a] Mean of 3 replicates; means in the same row followed by the same letter are not significantly different ($P > 0.05$); Duncan's multiple range test (SAS Institute 1985).

(SAN 810 I 1.3 GR). Each rate of application was made to separate pools on a preflood and postflood basis. Three test pools were used as untreated controls.

In field test 1, application rates of 2.8 kg/ha and 5.6 kg/ha were achieved by mixing 6.9 or 13.8 g, respectively, of the SAN 810 I 1.3 GR with 250 g (0.5 lbs) of sand in a 2-liter container 1 h before application. For preflood treatments, the appropriate sand-SAN 810 I 1.3 GR mixture was dispersed evenly by hand over the bottom of a test pool 10 days before the first flood/dry cycle. Pools were flooded with well water by opening a common valve connected by pipeline with a gate valve on each pool. The water level in each pool was maintained at 5 cm from the top by using an overflow pipe. Postflood treatments were made at the same rates of application as for the preflood treatments but by applying the sand-SAN 810 I 1.3 mixture evenly over the surface of a freshly flooded pool. There were 5 flood/dry cycles in field test 1.

In field test 2, the experimental design, treatment structure and application methodology were identical to those used in field test 1 except that only 4 flood/dry cycles were required to complete the test.

Data were analyzed using analysis of variance procedures (PROC ANOVA, SAS Institute 1985). Treatments comprised the rate of application and the timing (preflood vs. postflood) of application of SAN 810 I 1.3 GR. Data from each flood/dry cycle were analyzed independently.

RESULTS

In field test 1, SAN 810 I 1.3 GR applied as a preflood treatment at the rate of 2.8 kg/ha resulted in 93% E. I. for 1 flood/dry cycle (Table 1). Thereafter, % E. I. decreased to ≤58% for 2 flood/dry cycles and to <5% for cycles 4 and 5. When applied on a postflood basis at the rate of 2.8 kg/ha, SAN 810 I 1.3 GR resulted in 100% E. I. for 1 flood/dry cycle. Inhibition of emergence decreased to 58, 62, 4 and 4%, respectively, over the next 4 cycles. At 5.6 kg/ha, ≥90% E. I. was achieved with both the pre- and postflood treatments for 3 flood/dry cycles except for cycle 2 (E. I. = 87.3%) for the postflood treatment. For cycles 4 and 5, emergence inhibition was ≤10%.

In field test 2 (Table 1), <80% E. I. was obtained with both the pre- and postflood treatments at 2.8 kg/ha after the first flood. The preflood 5.6 kg/ha treatment resulted in ≥80% E. I. for the first flood/dry cycle only. The postflood 5.6 kg/ha treatment produced ≥92% E. I. for the first 2 flood/dry cycles, but only ≤40% E. I. thereafter. The percent E. I. was never greater than 12% for the check pools in either test.

Both the rate of application of SAN 810 I 1.3 GR and the timing of flooding significantly ($P < 0.05$) influenced percent E. I. in *Ae. taeniorhynchus* (Table 1). In field test 1, no statistically significant ($P > 0.05$) difference was detected in percent E. I. for either application rate between pre- and postflood treatments after the first 2 flood/dry cycles. Although not statistically different, there was a large average difference in the overall percent E. I. between the application rates. The lack of a statistical difference was probably due to the large variation in the percent E. I. among pools with the 2.8 kg/ha application rate. After the third flood/dry cycle, there was a statistically significant ($P < 0.05$) difference in the percent E. I. between the 2 application rates. In field test 2, there was a statistical difference between the 2 application rates, both pre- and postflood, after the first flood/dry cycle.

DISCUSSION

The higher application rate of SAN 810 I 1.3 GR provided more long-lasting and consistent control results than the lower rate. Causes for differences, especially in preflood treatments, between tests were not determined. The material used for treatment was from the same batch, and between tests it was stored in darkness at ca. 25°C. The occurrence of rainfall during the 10-day period beginning with the preflood treatment until the time pools were first flooded may be a factor. Overall, more frequent and heavier rainfall occurred during the preflood period of field test 2 than during the same period of field test 1. During the preflood interval, rain fell twice in field test 1, totaling 1.4 cm (0.5 in.), and 4 times during field test 2, totaling 7.3 cm (2.9 in.). Also during field test 2, it rained more frequently after the pools were flooded, and average ambient air temperature was several degrees higher (26.7 vs. 24.3°C) than during the field test 1. For example, by the end of the second flood/dry cycle, rain fell 5 times totaling 8.1 cm (3.2 in.) and 16 times totalling 20.6 cm (8.1 in.), respectively, for field tests 1 and 2. Although the pools never overflowed, rainfall may have diluted the effectiveness of the methoprene.

This formulation of methoprene at the 5.6-kg/ha rate of application effectively (>90% E. I.) controls *Ae. taeniorhynchus* as either a pre- or postflood treatment for 1–3 flood/dry cycles, depending on when the application was made. Floore et al. (1990) found that Altosid® pellets containing 4% methoprene effectively reduced *Ae. taeniorhynchus* emergence for more than 3 wk at application rates of 2.2- and 4.5-kg/ha in saltwater plots. The effectiveness of the 2.2-kg/ha rate was greatly reduced 22 days posttreatment, but the 4.5-kg/ha treatment was effective for 34 days.

Sustained-release formulations of larvicides may be a useful tool for saltmarsh mosquito control. Remote areas that are difficult to reach on a routine basis can be treated at the onset of the breeding season. The field life of formulations needs to be determined under natural weather conditions, e.g., when there is no rainfall, or frequent rainfall but with too little accumulation to induce egg hatch over extended periods of time.

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