SMALL PLOT FIELD TESTS OF METHOPRENE FOR THE CONTROL OF ASYNCHRONOUS BROODS OF *CULEX NIGRIPALPUS* THEOB. IN FLORIDA

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ABSTRACT. Two formulations of methoprene were applied at several dosages rates in 1 or 2 applications per week to asynchronous broods of *Culex nigripalpus* Theob. in replicated small plot field tests. Two applications per wk of a sand granular formulation at 0.022 kg AI/ha (0.020 lb AI/a) or 2 applications per wk of a water formulation at 0.030 kg AI/a(0.027 lb AI/a) were required for effective control.

Excellent control of *Aedes taeniorhynchus* (Wied.) has been obtained in Florida with 0.024 kg AI/ha (0.02 lb AI/acre) or less of methoprene either as a water or a sand formulation (Rathburn and Boike 1975 and 1977, Rathburn et al. 1978 and Rogers et al. 1976). Because of its importance as a vector of St. Louis encephalitis, the control of *Culex nigripalpus* Theob. is also of great importance in Florida. However, generally poor results have been obtained with methoprene against *Culex* species in Florida and elsewhere.

Case and Washino (1978) obtained only a 50% reduction in field emergence of *Cx. tarsalis* at the rather high dosage of 0.112 kg AI/ha (0.1 lb AI/acre). Self et al. (1978) obtained effective field control of *Cx. quinquefasciatus* for 3–5 wk after 1 application of a very high rate of 1 ppm. Dame et al. (1976), however, obtained effective field control of a mixed field population of predominately *Cx. nigripalpus* and Cx. *salinarius* after 3 days with an application of only 0.028 kg AI/ha (0.025 lb AI/acre) and the effect lasted for 5–6 days. Therefore, at best, highly variable results have been obtained in field tests of methoprene against *Culex* species.

In laboratory tests, Hsieh and Steelman (1974) also obtained wide variations in susceptibility to methoprene among 3 species of *Culex* as well as among 3 species of *Aedes,* but their results and the results of Rathburn and Boike (1975), Dame et al. (1976) and others demonstrate the reduced susceptibility of *Culex* species as compared to *Aedes, Anopheles* and *Psorophora* species. The following tests were conducted to demonstrate an effective dosage for the control of multiple broods of *Cx. nigripalpus.*

METHODS

The tests were conducted in a series of 8 specially constructed fresh water ponds. Each pond was 3m x 3m x 0.5m deep and was lined with polyethylene sheeting which was covered with a layer of grass sod 15 cm thick. Each plot was filled with fresh water to a depth of approximately 30 cm. Water was continuously added to each plot by means of individually controlled water supplies in order to maintain the desired water depth due to loss by evaporation, plant utilization and seepage.

Because of a large population of predaceous dytiscid larvae and dragonfly naiads in the test ponds, the larvae were placed in fine mesh nylon nets of 52 x 56 strands per in. The nets, each 60 cm square and 30 cm deep, were attached to a frame, constructed of 2 x 2 cm wood strips, and were floated by means of styrofoam floats placed at each corner. Several small stones were placed in each net to keep the bottom area of the net in contact with the bottom of the pond. Two nets were placed in each plot and several thousand laboratory-reared second instar larvae were added to each plot 2 to 4 times per wk. Pupae were sampled daily when present, placed in 250 ml glass beakers and set out-of-doors for adult

emergence. The present emergence and corrected percent control were determined as follows:

$$\% \text{ emergence} = \frac{\text{CS-DA}}{\text{CS+PE+DP}}$$

Where:

CS = Cast pupal skins
DA = Dead adults on water surface
PE = Partially emerged adults
DP = Dead pupae

$$\text{Corrected \% control} = \frac{(100-\text{\% treatment emergence}) - (100-\text{\% check emergence})}{\text{\% check emergence}}$$

Two formulations of methoprene, a sand granular formulation and a water formulation, were tested over a period of ca. 5 mo. The sand formulation was prepared by adding 9.3 g of Altosid SR-10 to 435.4 g of 20–45 mesh washed sand. The ingredients were stirred until uniformly mixed, then 4.5 g of HiSil 233 were added and again stirred until uniformly mixed. The dosages tested were 0.022 kg AI/ha (0.020 lb AI/acre) and 0.011 kg AI/ha (0.010 lb AI/acre) each applied twice a wk and 0.022 kg AI/ha (0.020 lb AI/acre) applied once a wk. The gross volume was 11.2 kg/ha (10 lb /acre) for the highest dosage and 5.6 kg/ha (5 lb/acre) for the lowest dosage. Two tests were conducted, the first for a period of 3 wk and the second for a period of 4 wk, and each test consisted of 2 replications of each dosage and the untreated check.

The water formulation was prepared by diluting 6.25 ml of Altosid SR–10 to 1000 ml of water. The number of ml of the dilution to give the desired dosage was then applied by means of a plastic clothes sprinkler to each plot. The dosages tested were 0.045 kg AI/ha (0.040 lb AI/acre), 0.030 kg AI/ha (0.027 lb AI/acre) and 0.015 kg AI/ha (0.013 lb AI/acre) each applied twice a wk and 0.030 kg AI/ha (0.013 lb AI/acre) applied once a wk. The gross volume per acre was 70 l/ha (7.5 gal/acre) for the highest dosage, 47 l/ha (5.0 gal/acre) for the middle dosage and 23 l/ha (2.5 gal/acre) for the lowest dosage. Three tests were conducted over a period of 11 wk. Since only 8 plots were available, each of the 3 treatments and an untreated check could not be conducted at the same time. The first test was conducted for a period of 3 wk and consisted of 2 replications each of the 0.030 kg AI/ha dosage treated once and twice a wk, the 0.015 kg AI/ha dosage treated twice a wk and the untreated check; the second test also conducted for a period of 3 wk consisted of 4 replications of the 0.015 kg AI/ha dosage applied twice a wk, 2 replications each of the 0.030 kg AI/ha dosage applied twice a wk and untreated check; and the third test, conducted for a period of 5 wk, consisted of 4 replications of the 0.045 kg AI/ha dosage applied twice a wk, 2 replications each of the 0.030 kg AI/ha dosage applied twice a wk and the untreated check.

Prior to the initial application of each dosage in each test, the length and width of the water surface of each plot was measured and the surface area of the plot in square feet was determined in order to apply the correct amount of each formulation. The same 2 ponds were always used for the untreated checks and where a dosage in a treated pond was changed, a higher dosage was always used and no pupae were sampled for emergence during at least 1 wk following a change in dosage. In the initial tests, the nets were removed from the test after the final treatment, washed with soap and water and allowed to air dry in direct sun light for a week before being reused. In later tests, the nets were removed weekly and cleaned in the same manner.

RESULTS

The results of the tests of the sand granular formulation of methoprene (Table 1) show that satisfactory control of asynchronous broods of *Cx. nigripalpus* was obtained in each test with two applications per wk of 0.022 kg AI/ha. Be-

Table 1. Small plot field tests of a sand formulation of methoprene for the control of asynchronous broods of *Culex nigripalpus* Theob. in Florida.

Test No.	Test duration wks.	Dosage-AI kg/ha	Dosage-AI lb/acre	No. trtmts. per wk.	No. of reps.	No. of pupae sampled	Avg. % emergence	Corrected % control
1	3	0.022	0.02	2	2	1349	2.4	97.3
		0.011	0.01	2	2	1189	19.8	77.5
		0.022	0.02	1	2	2460	55.7	36.8
		Check		—	2	1699	88.1	—
2	4	0.022	0.02	2	2	961	4.3	94.0
		0.011	0.01	2	2	1427	10.2	85.4
		0.022	0.02	1	2	1995	39.2	44.1
		Check		—	2	1601	70.1	—
Total	7	0.022	0.02	2	4	2310	3.2	96.0
		0.011	0.01	2	4	2616	14.5	81.7
		0.022	0.02	1	4	4455	48.3	39.1
		Check		—	4	3300	79.3	—

tween the first and second test, a sharp decline in temperature occurred. The water temperature during the 3 wk of the first test ranged from an avg daily minimum of 26°C to an avg daily maximum of 36°C and often exceeded 38°C. The water temperature during the 4 wk of the second test ranged from an avg daily minimum of 17°C to an avg daily maximum of 27°C. This change in temperature had no apparent effect on the % control of the various treatments. There was, however, a slight reduction in the avg % emergence of adults from the untreated check plots and in all the treated plots except for the highest dosages.

The results of the tests of the water formulation of methoprene are shown in Table 2. From these results, it is evident that almost perfect control of asynchronous broods of *Cx. nigripalpus* was obtained

Table 2. Small plot field tests of a water formulation of methoprene for the control of asynchronous broods of *Culex nigripalpus* Theob. in Florida.

Test No.	Test duration wks.	Dosage-AI kg/ha	Dosage-AI lb/acre	No. trtmts. per wk.	No. of reps.	No. of pupae sampled	Avg. % emergence	Corrected % control
1	3	0.030	0.027	2	2	250	0.8	99.1
		0.015	0.013	2	2	278	8.3	90.7
		0.030	0.027	1	2	492	18.1	79.6
		Check		—	2	1203	88.9	—
2	3	0.030	0.027	2	2	735	9.1	90.0
		0.015	0.013	2	4	2145	12.6	86.2
		Check		—	2	2008	91.2	—
3	5	0.045	0.040	2	4	2662	0.8	99.1
		0.030	0.027	2	2	2027	4.7	94.7
		Check		—	2	2596	88.4	—
Total	5	0.045	0.040	2	4	2662	0.8	99.1
	11	0.030	0.027	2	6	3012	5.4	94.0
	6	0.015	0.013	2	6	2423	12.1	86.6
	3	0.030	0.027	1	2	492	18.1	79.6
	11	Check		—	6	5807	89.5	—

[1] Average percent emergence and corrected percent control are based on average check emergence or mortality obtained only in the tests with the respective treatments.

with 0.045 kg AI/ha of the water formulation applied twice a wk and excellent control was still obtained with only 0.030 kg AI/ha applied twice a wk. The tests of the water formulations were conducted during June, July, and August when there was little change in daily termperatures. During this period, the avg daily maximum water temperature was 36°C and the avg daily minimum was 27°F.

Data obtained from Zoecon Corporation indicate that at 38°C, a water temperature not unlike that obtained in these tests, the % trans isomer inside the capsule of Altosid SR-10 was 65, 42, 26, 13, 4, and 0% after 1, 2, 3, 4, 5, and 6 days, respectively. Using these figures, the amount of toxicant present in the water each day from various treatments with the water formulation was calculated. These data are shown in Table 3.

Although the avg kg AI/ha for the 0.030 kg AI/ha dosage applied once a wk and the 0.015 kg AI/ha dosage applied twice a wk were the same for the 2-wk period shown, the number of days the avg kg AI/ha was less than 0.01 kg AI/ha was 8 days for the 0.030 kg AI/ha dosage applied once a wk as compared to 6 days for the 0.015 kg AI/ha dosage applied twice a wk. Also, the 8 days the former dosage was less than 0.01 kg AI/ha were grouped at two time intervals, whereas the 6 days the latter dosage was less than 0.01 kg AI/ha were spread out over the entire 2 wk period and for no more than 2 consecutive days. Better control was also obtained with 0.015 kg AI/ha applied twice a wk.

This effect is also evident with the 2 higher dosages tested where the 0.030 kg AI/ha dosage applied twice a wk resulted in only 2 days in which the dosage was below 0.01 kg AI/ha and gave an avg mortality of 94.0% and the 0.045 kg AI/ha dosage applied twice a wk resulted in no days in which the dosage was less than 0.01 kg AI/ha and gave an avg kill of 99.1%.

Although the dosage required to effect satisfactory kill of asynchronous broods of *Cx. nigripalpus* is considerably above

Table 3. Daily dosages of methoprene present in small plot tests treated once and twice per week with various dosages.

Day No.	% trans isomer inside capsule[1] 1 trt/wk	2 trt/wk	Daily dosages (kg AI/ha) present for indicated treatments 0.030 kg AI/ha once per week	0.015 kg AI/ha twice per week	0.030 kg AI/ha twice per week	0.045 kg AI/ha twice per week
1	100	100	0.030	0.015	0.030	0.045
2	65	65	0.020	0.010	0.020	0.029
3	42	42	0.013	0.006*	0.013	0.019
4	26	26+100	0.008*	0.019	0.038	0.056
5	13	13+65	0.004*	0.012	0.023	0.035
6	4	4+42	0.001*	0.007*	0.014	0.021
7	0	0+26	0.000*	0.004*	0.008*	0.012
8	100	100+13	0.030	0.017	0.034	0.051
9	65	65+4	0.020	0.010	0.021	0.031
10	42	42+0	0.013	0.006*	0.013	0.019
11	26	26+100	0.008*	0.019	0.038	0.056
12	13	13+65	0.004*	0.012	0.023	0.035
13	4	4+42	0.001*	0.007*	0.014	0.021
14	0	0+26	0.000*	0.004*	0.008*	0.012
Average kg AI/ha[2]			0.011	0.011	0.021	0.032
*No. days 0.01 kg AI/ha			8	6	2	0
Average % control			79.6	86.6	94.0	99.1

[1] Based on data supplied by Zoecon Corporation.

[2] Average dosage for the entire test period is essentially the same as that for the 2 week period shown above due to the regular treatment schedule used.

that necessary to control flood water *Aedes* and material costs are almost doubled, this research demonstrates that practical control of asynchronous broods of *Cx. nigripalpus* can be obtained with either of the 2 formulations of methoprene applied twice a wk. This is particularly important when control of *Cx. nigripalpus* is necessary to reduce encephalitis transmission in epidemic situations.

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GENETICS OF WHITE EYE IN *ANOPHELES CULICIFACIES*

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ABSTRACT. Genetic analysis of a white eye mutant in *Anopheles culicifacies* Giles indicates that it is sex linked, recessive in females and hemizygous in males. The data indicate that white eye is allelic to another sex linked mutant, rose eye.

Anopheles culicifacies Giles, an important malaria vector in the Indo-Pakistan subcontinent, has been under intensive investigation in our laboratory for the past few years. A number of mutants have been described (Sakai et al. 1977, 1979a,c), a few enzyme polymorphisms have been investigated (Ahmad et al. 1978), a polytene chromosome map has been prepared (Saifuddin et al. 1978), a number of translocations have been induced, isolated and characterized (Baker et al. 1978), and the chromosomes have been correlated to their respective linkage groups (Sakai et al. 1979b).

During routine culling of a sex-linked translocation strain, *T(X,3R)1* (Baker et al. 1978), which was maintained as a heterozygote by crossing phenotypically wild type females to rose (Sakai et al. 1977) males, a few females with pale rose eyes were found. These females were crossed to wild type males of the standard Sattoki strain and the resulting F_1 progeny consisted of wild type females and rose and white eyed males. In the next generation the white eyed males were crossed to their phenotypically wild type sisters and in some of the resulting families both white eyed females and