

# EFFECT OF THE INSECT GROWTH REGULATOR METHOPRENE ON THE OVIPOSITIONAL BEHAVIOR OF *Aedes aegypti* AND *Culex quinquefasciatus*

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**ABSTRACT.** Laboratory and field tests were conducted to determine the attractiveness of the insect growth regulator methoprene to ovipositing mosquitoes when presented at concentrations used in mosquito control programs. Laboratory experiments indicated that ovipositing *Culex quinquefasciatus* and *Aedes aegypti* were not attracted to methoprene. In field experiments, *Culex stigmatosoma* and *Cx. quinquefasciatus* larval dip counts in experimental ponds treated with methoprene briquets were not significantly different from untreated control ponds. Water taken from these methoprene-treated ponds was not attractive when compared with water taken from untreated ponds to ovipositing *Cx. quinquefasciatus* in laboratory experiments. These studies provide strong evidence that methoprene is not attractive to ovipositing mosquitoes at concentrations within the range of field applications.

## INTRODUCTION

Various chemical compounds may serve as ovipositional attractants/stimulants or repellents for mosquitoes even when present in relatively small quantities. Millar et al. (1992) showed that *Culex quinquefasciatus* Say responds and oviposits preferentially in waters containing as little as 10 parts per trillion of 3-methylindole. Larvicidal compounds used for mosquito control are applied at concentrations that are well above this concentration, yet there has been little research on the effect of these larvicidal compounds on the ovipositional behavior of gravid mosquitoes.

Oviposition traps containing a very high concentration of emulsifiable chlorpyrifos were repellent to ovipositing *Aedes triseriatus* (Say), while granular formulations of chlorpyrifos and temephos were not repellent (Mather and DeFoliart 1983). These data suggested that the differences in oviposition trap water were due to the solvents and additives in the emulsifiable concentrate rather than the active ingredients themselves. In another field study, malathion and temephos were at relatively high concentrations repellent to *Aedes aegypti* (Linn.) in oviposition traps (Moore 1977). At much lower concentrations, cypermethrin, fenvalerate, decamethrin and permethrin have been demonstrated to have some repellent effect on gravid *Anopheles stephensi* Liston, *Ae. aegypti* and *Cx. quinquefasciatus* under laboratory conditions (Verma 1986). Carroll (1979) reported that oviposition traps treated with pellets containing the insect growth regulator (IGR) methoprene were more attractive to ovipositing *Ae. aegypti* in field studies than traps that contained only tap water.

The objective of this study was to determine through controlled laboratory and field experiments if methoprene applied at field rates, has

a consistent and repeatable effect on the ovipositional response of *Ae. aegypti* and *Cx. quinquefasciatus*.

## MATERIALS AND METHODS

**Laboratory experiments:** Two-choice laboratory experiments that assessed the response of gravid female mosquitoes to methoprene-treated water were conducted in 23 × 23 × 32 cm cages housed in a photoperiod (14L:10 D including a 2 h evening twilight period) and temperature (24 ± 3°C) controlled insectary. Each cage contained 2 randomly placed waxed paper cups (5 × 7.5 cm diam), one treatment cup containing 80 ml distilled water with methoprene and the other without methoprene serving as a control. A total of 6 cages were employed simultaneously for each experiment. Twenty gravid (7–10 days post-bloodmeal), laboratory-reared *Cx. quinquefasciatus* or 15 or 20 *Ae. aegypti* were placed in each cage at 1600 h and left undisturbed overnight. The following morning (0800 h) the number of egg rafts in each cup was recorded. The data was square root transformed ( $\sqrt{x}$ ) to correct for unequal variances (Box et al. 1978) and paired *t*-tests were performed. Each experiment using s-methoprene or methoprene pellets was repeated 3 times for a total of 18 replicates.

Following the above protocol in experiment 1, one ppm AI (active ingredient) (a concentration close to that used in the field) technical grade s-methoprene (Lot # R-277-105-1, Zoecon Corporation, Dallax, TX) in 25  $\mu$ l ether was diluted in the ovipositional cup water, and its attractancy to gravid *Cx. quinquefasciatus* was compared with cups containing distilled water and solvent only. In experiment 2, the attractancy of methoprene pellets to gravid *Cx. quinquefasciatus* was evaluated by comparing the oviposition in test cups containing distilled water and

a single methoprene pellet (Altosid® Pellets, Lot # 91040401, Zoecon Corporation, Dallas, TX) against cups containing water and blank pellets (control). The resulting concentration of methoprene in these experiments was established to be a maximum of 5 ppm AI, based upon a mean pellet weight of 0.148 g ( $n = 100$ ) and a theoretical complete release of methoprene from the pellet.

The next 2 experiments assessed the attractiveness of distilled water containing methoprene pellets to gravid *Ae. aegypti*. Experiment 3 followed the same pellet test protocol as that provided above with some modification (20 mosquitoes/cage). Since *Ae. aegypti* oviposits single eggs on the walls of containers rather than in rafts, a filter paper disk (9 cm diam, Whatman Qualitative #2) cut in half was placed along the side of each oviposition cup to serve as an oviposition substrate. Experiment 4 was performed to determine the effects of methoprene age within an oviposition trap on ovipositional behavior. Six oviposition traps (350 ml aluminum beverage cans with the tops removed) were filled with distilled water and treated with either a methoprene pellet or a blank pellet and placed in the field for 48 h (ambient temperatures 10–24°C). Water samples taken from the traps were compared for oviposition (15 gravid *Ae. aegypti*/cage) in the laboratory as above. As single eggs on filter paper were difficult to accurately count if too numerous, fewer gravid *Ae. aegypti* were used in experiment 4.

**Field experiments:** The effect of methoprene on oviposition in the field was also considered. Eleven wood-sided experimental ponds (27 m<sup>3</sup>) at the University of California-Riverside Aquatic and Vector Control Research Facility in Riverside, CA were flooded to a mean depth of 30 cm with canal water on October 31, 1991. Two days post-flooding all experimental ponds were treated with bifenthrin (0.5 g/h AI) to control tadpole shrimp [*Triops longicaudatus* (LeConte)]. Predation by shrimp reduces populations of larval *Culex* mosquitoes (Walton et al. 1990) and the presence of shrimp may also negatively influence oviposition by *Culex* (Tietze and Mulla 1991). Mulla et al. (1992) found that doses of bifenthrin in the range of 0.25 to 0.5 g/h AI will control the shrimp, but will not have a negative effect on mosquito larval populations.

A completely randomized block design was used to assign methoprene treatments to experimental ponds. Three days post-flooding, 4 experimental ponds were treated with methoprene briquets (Altosid® XR Briquets, Lot # 9H016, Zoecon Corporation, Dallas, TX) at 1× the labeled field rate (2 briquets/pond), 4 ponds at 2× (4 briquets/pond) the labeled rate for metho-

prene and 3 experimental ponds served as untreated controls. Five and 7 days post-treatment, five dip samples (350 ml ea) were taken in each pond, 1 in each corner and 1 along the center of the north side rail. The larval dip counts between the treated ponds and the controls were compared using ANOVA to determine if there were differences in larval densities, which may be indicative of differential oviposition.

Water samples were collected in glass containers from the control and from the 1× treatment ponds at 5, 9 and 11 days post-treatment for laboratory oviposition experiments, comparing each for attractancy or repellency to gravid *Cx. quinquefasciatus* using similar laboratory methodology as described above. Water samples taken from ponds treated at 2× the field rate and the controls at 5 and 8 days post-treatment were tested similarly in the laboratory.

## RESULTS AND DISCUSSION

**Laboratory experiments:** All 4 laboratory experiments failed to show any attractancy or repellency of *Cx. quinquefasciatus* or *Ae. aegypti* to technical, pellet or briquet methoprene at the normal application rate. Data from experiment 1, which compared the attractancy to ovipositing *Cx. quinquefasciatus* of 1 ppm AI technical methoprene with distilled water controls are summarized in Table 1. The mean number of egg rafts collected per cup was 4.7 and 6.2 in the test and the control cups, respectively. Paired *t*-tests on square root transformed data showed no significant difference between cups containing distilled water treated with methoprene and distilled water controls ( $df = 17$ ;  $P > 0.05$ ).

Experiment 2 compared the oviposition attractancy of methoprene pellets to gravid *Cx. quinquefasciatus* compared with distilled water in cups containing water and blank pellets (Table 1). Oviposition cups containing a methoprene pellet received a mean of 5.3 egg rafts/cup and cups containing distilled water with a blank pellet received a mean of 5.0 egg rafts/cup. Paired *t*-tests based on square root transformed data showed no significant difference between these tests and controls ( $df = 17$ ;  $P > 0.05$ ).

The following experiments examined the effect of methoprene on the oviposition behavior of *Ae. aegypti* in the laboratory (Table 1). In experiment 3, distilled water containing a methoprene pellet received a mean of 304.1 eggs/filter paper strip, and distilled water containing a blank pellet received a mean of 297.4 eggs/filter paper strip. Paired *t*-tests on square root transformed data showed that there was no significant difference in the number of eggs re-

ceived on the filter paper among the tests or controls ( $df = 17$ ;  $P > 0.05$ ). Aged methoprene-treated water removed from oviposition traps were tested for their attractancy/repellency in laboratory experiments. Water taken from oviposition traps that contained methoprene pellets received a mean of 61.6 eggs/filter paper strip while water taken from traps that contained blank pellets received a mean of 62.2 eggs/filter paper strip. There was no significant difference between the number of eggs deposited on the strips in the water taken from traps that contained methoprene pellets and water taken from traps that contained blank pellets ( $df = 17$ ;  $P > 0.05$ ).

**Field experiments:** The final group of experiments, waters taken from methoprene briquet-treated experimental ponds, were analyzed for their attractancy to ovipositing *Culex* mosquitoes in 2 ways. First, larval dip samples were taken 5 and 7 days post-treatment from ponds which had been treated at 1× and 2× the field rate of methoprene briquets, as well as the untreated controls. Five days post-treatment, a mean of 17.3/dip L3-L4 *Cx. quinquefasciatus* and *Culex stigmatosoma* Dyar larvae were collected from untreated ponds (Fig. 1). In experimental ponds that were treated at the label rate (1×), 17.6 larvae/dip were collected and in ponds treated at 2×, 29.4 larvae/dip were collected. The high mean value on day 5 for the 2× treatment is explained by exceptionally high counts in 1 pond. At 7 days post-treatment, a mean of 14.8 larvae/dip were recovered from control ponds, 12.9 larvae/dip from 1 × methoprene briquet ponds and 15.7 larvae/dip from ponds treated with 2× methoprene. An ANOVA of the square root transformed count data showed that there was no significant difference between the number of larvae sampled from experimental ponds treated with methoprene at either concentration or untreated ponds for 5 or 7 days post-treatment ( $df = 2,6$ ;  $P > 0.05$ ).

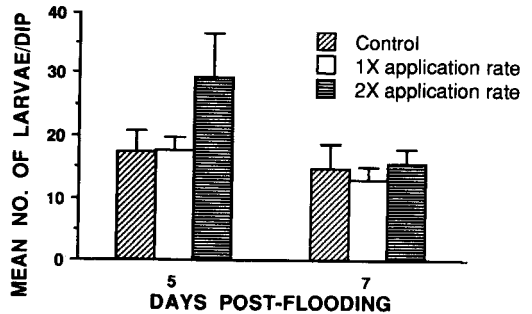


Fig. 1. Influence of methoprene briquets on larval populations of *Culex quinquefasciatus* and *Cx. stigmatosoma* in experimental ponds at 1× and 2× the labeled treatment rate. There was no significant difference between treatments ( $P > 0.05$ ).

Second, oviposition-choice experiments were conducted in the laboratory with *Culex quinquefasciatus* using water samples taken from the experimental ponds treated with methoprene and those left untreated. Water samples from ponds containing methoprene at 1× or 2× the field rate were not significantly different ( $df = 5$ ;  $P > 0.05$  for each test) from water taken from control ponds in attractancy to ovipositing *Cx. quinquefasciatus* on any day sampled (Table 2).

In all of the laboratory and field experiments conducted, methoprene in technical, pellet or briquet formulations did not show significant attraction to ovipositing *Cx. quinquefasciatus* or *Ae. aegypti* mosquitoes. These results are in direct contradiction to those of Carroll (1979), who reported through field studies that oviposition traps containing methoprene "minikets" received more *Ae. aegypti* eggs than those that contained plain tap water. The apparent attractancy reported by Carroll may have been due to degradation of the briquet itself, which was not accounted for since a blank briquet was not used as a control. A decomposing briquet may alter the physical features (color, light reflectance) of

Table 1. Effect of methoprene on the oviposition behavior of *Culex quinquefasciatus* and *Aedes aegypti* as assessed in the laboratory.

Species	Exp.	Mean no. $\pm$ SE of egg rafts/cup	
		Methoprene	Control
<i>Cx. quinquefasciatus</i>	1 <sup>1</sup>	4.8 $\pm$ 0.93 <sup>2</sup>	5.7 $\pm$ 1.1 n.s.
<i>Cx. quinquefasciatus</i>	2	5.1 $\pm$ 0.7 <sup>3</sup>	5.3 $\pm$ 0.9 n.s.
<i>Ae. aegypti</i>	3	287.7 $\pm$ 48.5 <sup>3</sup>	290.0 $\pm$ 26.6 n.s.
<i>Ae. aegypti</i>	4	62.5 $\pm$ 20.1 <sup>4</sup>	63.9 $\pm$ 18.1 n.s.

<sup>1</sup> Experiments 1-3 consisted of 18 replicated cages with 20 gravid females/cage. Experiment 4 consisted of 18 replicated cages with 15 gravid females/cage.

<sup>2</sup> Technical grade s-methoprene (1 ppm).

<sup>3</sup> Altosid pellet (5 ppm).

<sup>4</sup> Water taken from oviposition traps held in field for 48 h for each experiment.

n.s. = not significant ( $P > 0.05$ ).

Table 2. Effect of water from methoprene briquet-treated experimental ponds on the oviposition behavior of *Culex quinquefasciatus* in the laboratory.

Days post-treatment	Rate <sup>1</sup>	Mean no. $\pm$ SE of egg rafts/cup	
		Methoprene	Control
5 <sup>2</sup>	1 $\times$	14.3 $\pm$ 1.6	9.0 $\pm$ 1.2 n.s.
5	2 $\times$	6.5 $\pm$ 2.3	5.5 $\pm$ 1.5 n.s.
8	2 $\times$	8.2 $\pm$ 2.2	4.5 $\pm$ 1.2 n.s.
9	1 $\times$	7.8 $\pm$ 1.4	9.0 $\pm$ 1.6 n.s.
11	1 $\times$	6.3 $\pm$ 1.6	6.5 $\pm$ 1.1 n.s.

<sup>1</sup> Refers to the label rate for stagnant water mosquitoes.

<sup>2</sup> Each experiment consisted of 6 replicated cages with 20 gravid females/cage.  
n.s. = not significant ( $P > 0.05$ ).

the water, which have also been reported to attract mosquitoes (Wallis 1954, Beehler and DeFoliart 1990). Through the experiments herein reported, we feel that there is insufficient evidence to support the contention that methoprene is attractive to ovipositing mosquitoes when presented in concentrations that are used in the field.

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