EFFECTS OF THE INSECT GROWTH REGULATOR METHOPRENE ON NATURAL POPULATIONS OF AQUATIC ORGANISMS IN LOUISIANA INTERMEDIATE MARSH HABITATS

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ABSTRACT. A study was conducted in a Louisiana coastal marsh to determine the effects of the insect growth regulator methoprene on aquatic organism populations.

Six aerial applications of methoprene (28 gm AI/ha) during an 18-month period caused statistically significant differences in certain aquatic population, when treated and untreated populations were compared. Methoprene caused highly significant ($P < 0.01$) reductions in natural populations of the scud, *Hyalella azteca* (Saussure) adults and young; opossum shrimp, *Taphromysis louisianae* (Banner) adults and young; freshwater prawns, *Palaemonetes paladosus* (Gibbs) adults and young; mayflies, *Callibaetis* sp. naiads; dance flies, *Notophila* sp. larvae; midges, Chironomidae larvae; fresh water snail, *Physa* sp. adults and young; damselflies and dragonflies, *Enallagma, Anax* and *Belonia* spp. naiads; burrowing water beetles, *Suphisellus* sp. adults and *Hydrocanthus* sp. adults; and water scavenger beetles, *Berosus infuscatus* Leconte adults and *Berosus* spp. larvae. Populations of the water boatmen, *Trichocorixa louisianae* Jaczewaski nymphs; moth flies, *Psychoda* sp. larvae; crawfish, *Procambarus clarki* (Girard) and *Cambarellus* sp. adults and young and predaceous diving beetle, *Liodessus affinis* (Say) adults significantly ($P < 0.05 - 0.01$) increased after the methoprene applications. No statistically significant ($P > 0.05$) difference was determined between the population numbers of 28 aquatic organisms when treated and untreated populations were compared.

The data indicated that of those populations significantly reduced by the methoprene applications, 7 organisms were collected in significantly greater numbers from the habitat containing emergent vegetation, 2 organisms were more abundant in the open water habitat and 5 organisms were collected in almost equal numbers from both habitats. Those organisms not affected by the methoprene treatment were collected in greater numbers from the vegetative habitat (24) followed by the open water habitat (6) and 3 organisms showed no preference for either habitat.

The development of resistance to organophosphate compounds by various mosquito species has necessitated the use of alternative chemicals. One such alternative is the use of insect growth regulators (IGR). Several studies have shown the value and operational feasibility of the IGR methoprene [isopropyl (2E, 4E) -11-methoxy -3, 7, 11 - trimelthyl -2, 4 - dodecadienoate] for the control of mosquito larvae (Dunn et al. 1974, Lewallen and Ramke 1974, Schaefer et al. 1974, Mulla and Darwazeh 1975 a and b, Rathburn and Boike, 1975 and Steelman et al. 1975).

The acute effects of methoprene on non-target organisms have been demonstrated (Miura and Takahashi 1973, Dunn et al. 1974, Miura and Takahashi 1974, Schaefer et al. 1974 and Takahashi and Miura 1975). Norland and Mulla (1975) in a 3-month study measured changes in bionomics and abundance of several non-target organisms after treatment with methoprene. Steelman et al. (1975) reported that the effects of methoprene on a *Tropisternus* sp. larval population was not evident until the adult stage of the insect was reached.

This paper contains the results of a

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study conducted to determine the long range impact of methoprene on populations of non-target aquatic organisms in Louisiana marsh habitats.

MATERIALS AND METHODS

Two 20 ha adjoining plots were selected in an intermediate marsh (Chabreck 1972) near Grand Chenier in Cameron Parish, Louisiana. One 20 ha plot was treated with methoprene at a rate of 28 gm AI/ha by a Cessna Ag Wagon aircraft flying at 190 kph at an altitude of 6.1 m utilizing 18.3 m swaths. The treated plot received aerial applications of methoprene on June 11, September 20, 1974 and January 21, April 17, June 13, and September 15, 1975. The other 20 ha plot was left untreated for population density comparison.

Samples were collected at ca 2 wk intervals throughout the 18 mo study. Ten 0.09 m2 (1 ft^2) samples were taken randomly from as near to the center of the plots as conditions allowed. The 10 samples consisted of 5 taken in open water areas and 5 taken in areas having abundant emergent vegetation.

A sampling device was constructed of 3.18 mm (⅛") sheet metal and resembled a 0.0283 m^3 (1 ft^3) box open at both ends and was designed to sample 0.09 m^2 (1 ft^2) of marsh. Angle iron (12.7 mm) was used to reinforce the upper edges of the device and a band of angle iron was welded to the outside 7.62 cm above the bottom. The lower edges were sharpened to facilitate cutting through the sod and substratum. The lower band of angle iron insured the uniform implantation of the device to a depth of 7.62 cm into the soil.

Samples were taken by randomly placing the sampler into the marsh to a depth of 7.62 cm. The water depth was measured inside the sampler and the vegetation within the sample pulled up and washed in the sample water. The top 2.54 cm of debris and mud was then agitated to facilitate collection of benthic organisms. The water was immediately removed from the sampler by means of a rectangular 1-liter can and filtered through a 100 mesh bag. The resulting mass of debris and organisms was placed in 95% ethyl alcohol in 1.1 liter mason jars and transported to the laboratory. Additionally, water and soil temperature and pH were measured at the site on each collection date.

STATISTICAL ANALYSIS

The statistical design used in this study was a 29x2x2x5 factorial in a completely randomized design. Data were statistically analyzed and analyses of variance were obtained with respect to the following: date, area (control vs treated); subarea (emergent vegetation vs open water habitats) and all possible interactions. A standard transformation ($\sqrt{X+1}$) was performed on the data to facilitate analysis. Correlation coefficients were also computed for each variable.

RESULTS AND DISCUSSION

A vegetative transect through the treated and control plot and salinity determinations made during the study showed the site to be intermediate marsh (Chabreck 1972). Salinity values ranged from 1.7–3.5 parts per thousand (ppt) with an average of 2.6 ppt in the treated and control plots. Water depths fluctuated throughout the year although there was no movement of water between the plots. Water depths in the plots varied on a south to north elevation gradient, the southern end the shallower, the northern end the deeper. This was substantiated by the vegetative types identified from these areas. The control plot water depth ranged from 3.81–25.40 cm (13.30 cm avg.) for the vegetative habitat and 6.35–29.21 cm (17.80 cm avg.) for the open water habitat and in the treated plot, ranged from 5.08–24.40 cm (12.47 cm avg.) for the vegetative habitat and 5.08–27.94 cm (16.84 cm avg.) for the open water habitat.

A severe drought occurred throughout the marshland during July and August, 1974. The entire marsh area was dry including the 2–20 ha test plots.

With the exception of muscid larvae a

highly significant ($P < 0.01$) difference existed between collection dates, probably attributable to variations in biological and seasonal cycles and environmental conditions of the plots at the time of collection.

A highly significant reduction ($P < 0.01$) occurred in populations of the scud *Hyallela azteca* (Saussure) when the treated and untreated plots were compared (Table 1). However, several investigators have tested the effects of methoprene on food chain crustaceans, but none has reported adverse effects on *H. azteca* caused by this chemical (Miura and Takahashi 1973, 1974, Norland and Mulla 1975).

The methoprene applications caused 37% reduction in the number of scuds/0.09 m^2. The analysis of variance of these data showed a highly significant ($P < 0.01$) area (treatment vs control) X subarea (vegetative vs open) interaction which indicated that the greater mortality occurred in the scud population in the treated open habitat (Table 1). This became evident when the scud populations in vegetative habitats of both treated and untreated areas were compared. The open-control area had significantly higher populations of scuds than the open-treated area.

Methoprene caused a highly significant ($P < 0.01$) reduction in the population numbers of the opossum shrimp *Taphromysis louisianae* (Banner) when the treated and untreated populations were compared. Significantly ($P < 0.01$) greater numbers of *T. louisianae* were collected from the open water habitats in both the control and treated plots. The treated opossum shrimp population was reduced by 84% when compared to the untreated control with slightly higher mortality occurring in the emergent plant habitat (89%) than in the open water habitat (83%).

During the 18-month study, the applications of methoprene significantly ($P < 0.01$) reduced the *Callibaetis* sp. naiad population (32%). Significantly ($P < 0.01$) more naiads were collected from the habitat that contained plants in the treated plot, while populations in the untreated plot were approximately the same in both habitats. Methoprene caused a 74% reduction in the naiad population in the open water habitat of the treated plot.

Table 1. Populations of aquatic organisms significantly ($P < 0.01$) reduced after exposure to 6 applications of methoprene (28 gm AI/ha/application over an 18 month period in a Louisiana intermediate marsh.)

Species	$\overline{X}$ No. Organisms Per $0.09m^2$					
	Control			Treated		
	Plants vs	Open	$\overline{X}$	Plants vs	Open	$\overline{X}$
Hyallela azteca A&I [a]	5.42 [b]	3.87	4.64 [b]	4.38 [b]	1.46	2.92
Taphromysis louisianae A&I	0.39	3.75 [b]	2.07 [b]	0.04	0.62 [b]	0.33
Callibaetis sp. I	0.59	0.65	0.62 [b]	0.66 [b]	0.17	0.42
Coenagrionidae I	1.83 [c]	1.28	1.56 [b]	0.97 [c]	0.76	0.87
Belonia & *Anax* I	0.15	0.10	0.12 [b]	0.02	0.02	0.02
Noteridae I	1.23 [b]	0.39	0.81 [c]	0.79 [b]	0.29	0.54
Hydrocanthus spp. A	0.35 [b]	0.14	0.24 [b]	0.13	0.14	0.13
Berosus infuscatus A	0.88	1.10 [b]	0.99 [b]	0.61 [b]	0.32	0.46
Berosus sp. I	4.01	4.32 [c]	4.16 [b]	1.14	1.12	1.13
Notophila sp. I	6.30 [b]	1.0	3.65 [b]	3.19 [b]	0.38	1.78
Chironomidae I	7.12	32.14 [b]	19.32 [b]	4.66	22.0 [b]	13.30
Physa sp. A&I	0.61 [b]	0.17	0.39 [b]	0.30 [b]	0.08	0.19
Palaemonetes paludosus A&I	0.06	0.69 [b]	0.38 [b]	0.01	0.01	0.01

[a] A = adult, I = immature.
[b] Significantly different ($P < 0.01$).
[c] Significantly different ($P < 0.05$).

Miura and Takahashi (1973) reported no effect on *Callibaetis* sp. when exposed to methoprene in the laboratory or in field studies. They also reported no effects were evident on the Odonata naiads *Triops longicaudatus* LeConte and *Argin* sp. It should be noted, however, that laboratory tests on these species were terminated after 48–72 hr. Schaefer et al. (1974) reported no effect on *Callibaetis* sp. after treatment with methoprene. Others have made similar reports with respect to *Callibaetis* sp. immatures (Schaefer et al. 1974) Steelman et al. 1975). Norland and Mulla (1975) observed that methoprene caused mortality to both early and late nymphal stages of *Callibaetis pacificus* Seeman in field tests and in the laboratory.

The methoprene treatments caused a highly significant ($P < 0.01$) reduction in the population numbers of coenagrionid naiads. Significantly ($P < 0.05$) more naiads were collected in the habitats that contained emergent vegetation in both the treated and the untreated plot. Methoprene caused 47 and 40% reductions in the naiad populations of the habitat that contained emergent plants and the open water habitats, respectively.

Miura and Takahashi (1973) reported no mortality to immature coenagrionids, *Argia* sp. after exposure to 1.0 ppm of methoprene for 48 hr in the laboratory. However, Steelman et al. (1975) reported a significant reduction in libellulid immatures after exposure to methoprene at rates used for mosquito control.

The treated populations of *Belonia–Anax* spp. were significantly ($P < 0.01$) reduced by the methoprene treatments during the 18 month period. No significant ($P > 01.05$) habitat preference was shown by the *Belonia* and *Anax* spp. naiads in either the treated or untreated plots and an 83% reduction occurred in the naiad populations of both habitats in the treated plot.

Approximately 13 days after the first methoprene application, both the control and treated noterid immature populations decreased with the treated population being significantly ($P < 0.01$) reduced when compared to the untreated control. Following the 2 month drought, no noterid immatures were collected in the treated plot on September 6, 1974; however, 14 days later, on September 9, 1974, the treated plot had significantly ($P < 0.01$) greater numbers of noterid immatures than the control plot. A methoprene application was applied on September 20, 1974, and the samples collected on October 4, 1974 showed that the noterid immatures had been significantly ($P < 0.01$) reduced in the treatment plot. From November 6, 1974, to April 14, 1975, no noterid immatures were collected from either the control or treated plots. A methoprene treatment was applied to the marsh on April 17, 1975, and we assumed that no noterid immatures were present at that time. The assumption that populations were not exposed to methoprene is substantiated by the fact that in 3 of 4 collection dates between May 1 and June 12, 1975, the treated plot populations were significantly higher ($P < 0.01$) than the control populations. Following the methoprene application of June 13, 1975, the treated population was significantly ($P < 0.01$) reduced when compared to the control population.

The methoprene treatments caused greater mortality in the open habitat when the 2 habitats from the treated and untreated plots were compared. Over the 18 month study, application of methoprene to the marsh ecosystem caused a significant ($P < 0.05$) reduction (33%) in the noterid immature population.

A highly significant ($P < 0.01$) reduction in the *Hydrocanthus* spp. adult population occurred after exposure to methoprene when compared to the control populations. Following the first application of methoprene on June 11, 1974 there was significantly ($P < 0.01$) more *Hydrocanthus* spp. adults in the treated plot when compared to the control plot. No collections were made during the 2 month drought of July and August, 1974 and at the time of the second application of methoprene on September 20, 1974, no adults were collected. Fourteen days later, on October 4, 1974, there were significantly more collected in the control plot than in the treated plot. From October 18, 1974 to May 1, 1975, only one collection date yielded any specimens, that on December 19, 1974. When specimens were again collected on May 13, 1975, there was no statistically significant ($P > 0.01$) difference between the control and treated populations. However, 39 days after the April 17 methoprene application, there were significantly fewer *Hydrocanthus* spp. adults collected from the treated plot when compared to the control plot.

Significantly ($P < 0.01$) more mortality occurred in the treated-vegetative habitat (63%) since no difference existed between the control-open water habitats and the treated-open water habitats. Over the 18 month study, methoprene application caused a 46% reduction in the number of *Hydrocanthus* spp. adults in the treated plot.

Both the adult and immature populations of *Berosus* sp. were significantly ($P < 0.01$) reduced after exposure to the methoprene treatments. The treated plot had significantly ($P < 0.01$) larger numbers of *Berosus infuscatus* LeConte adults when compared to the control prior to the July-August, 1974 drought. On the second collection date following the drought, September 20, 1974, no statistically significant difference was detected between the control and treated populations. Following a methoprene application on September 20, 1974, treated populations sampled 14 days later were significantly ($P < 0.01$) reduced when compared to the control and remained so until January 21, 1975. From January 21 to February 18, 1975, no *B. infuscatus* adults were collected from either plot. On May 1, 1975, 14 days after a methoprene application, no statistically significant ($P > 0.01$) difference was detected between the treated and untreated populations. From August 1, 1975, until the termination of the study, no *B. infuscatus* adults were collected from either plot.

After the first application of methoprene to the marsh ecosystem, populations of *Berosus* spp. larvae in the treated plot were significantly ($P < 0.01$) reduced when compared to the control. However, this may have been due to environmental conditions of the plots since small numbers of larvae were collected from both the treated and untreated plots at this time. On the collection date immediately following the July-August drought, significantly more *Berosus* spp. larvae were collected from the control plot when compared to the treated. On September 20, 1974, methoprene was applied and combined with the ensuing applications, significantly reduced the immature population of *Berosus* spp. in the treated plot over the 18 month study when compared to the treated population.

Significantly ($P < 0.01$) more *B. infuscatus* adults were collected from the open water habitat of the control plot while significantly ($P < 0.01$) more were collected from the vegetative habitat in the plot that received methoprene treatments. Significantly ($P < 0.05$) more *Berosus* sp. larvae were collected from the open water habitats of the control plot and as a result of the mortality caused by the methoprene treatments in both habitats of the treated plot, no significant ($P > 0.05$) difference existed between the *Berosus* sp. larval populations collected from the two habitats in the treated plot.

Steelman et al. (1975) reduced the population of another hydrophilid, *Tropisternus* spp., by treatments with methoprene. In their study, larvae were initially treated and the effects of the treatment showed up 80 days later in the adult population. In short-term laboratory and field tests, no adverse effects were observed on populations of *Tropisternus lateralis* (Fabricius) and *Hydrophilus triangularis* Say, both hydrophilids (Miura and Takahashi 1973, 1974, Dunn et al. 1974, Schaefer et al. 1974). In no case did these studies last for more than 10 days. Miura and Takahashi (1973) obtained 57% mortality to *Helophorus* sp. exposed to 0.8 ppm methoprene for 72–97 h. However, they reported no visible effects on *Helophorus* sp. in irrigated pastures that had been treated with methoprene.

Populations of chironomid and *Notophila* sp. larvae were significantly ($P < 0.01$) reduced after treatment with methoprene. This was confirmed by the results obtained by several workers. Miura and Takahashi (1974) reported that 70 and 50% mortality occurred in *Brachydertera agrentala* (Walker) and *Chironomus stigmaterus* Say populations, respectively, after they had been exposed to methoprene at a rate of 0.01 ppm in the laboratory. Miura and Takahashi (1974) and Schaefer et al. (1974) reported that chironomid immatures were "adversely affected" by methoprene. Steelman et al. (1975), however, collected significantly ($P < 0.01$) more chironomid immatures in plots that had been treated with methoprene than in untreated plots. They reported a significant ($P < 0.01$) negative correlation between chironomid immatures (prey) and libellulid immatures (predators).

On two of the 3 collection dates prior to the first methoprene application, the treated plot had significantly ($P < 0.01$) more chironomid larvae. Thirteen days after the 1st methoprene application, the treated population was significantly ($P < 0.01$) reduced when compared to the untreated control.

Following the 2 month drought, the treated plot population was significantly greater than the untreated population. Fourteen days after the 2nd methoprene application, the treated population had been significantly reduced when compared to the control population. During the colder months, both the treated and control populations decreased, however, the treated population numbers were significantly ($P < 0.01$) lower than those of the untreated population.

Over the 18 month period the methoprene treatments caused significant ($P < 0.01$) reductions in the chironomid immature population when compared to the control. Significantly ($P < 0.01$) more chironomid larvae were collected from the open water habitats of both plots throughout the 18 month study. The methoprene treatments caused almost the same percent reduction of chironomid larvae in the open water habitat (32%) as in the vegetative habitat (35%) of the treated plot.

Thirteen days after the first methoprene application, the treated population of *Notophila* sp. larvae was significantly ($P < 0.01$) reduced when compared to the control population. On the 1st collection date following the drought of July and August, 1974, no significant ($P < 0.01$) difference existed between the treated and control populations. After the September 30, 1974, methoprene treatment was applied the treated population was significantly ($P < 0.01$) lower than the control population. Thereafter, four additional applications of methoprene were made which resulted in a significant ($P < 0.01$) reduction in the treated population when compared to the control.

Approximately 50% of the *Notophila* sp. larvae were killed in both habitats of the treated plot. Methoprene caused 49% mortality to the larvae in the vegetative habitat and 62% mortality in the open water habitat.

The treated population of the physid snail (*Physa* sp.) was significantly ($P < 0.01$) reduced when compared to the untreated population. However, Miura and Takahashi (1973, 1974) reported no mortality to *Physa* sp. when treated with 100 ppm of methoprene for 72 hr.

Prior to the 1st methoprene application, the control plot had significantly ($P < 0.01$) more *Physa* sp. than the treated plot. Fourteen days following the 1st application, no *Physa* sp. were collected from the treated plots. During the period of September 6 to December 19, 1974, *Physa* occurred sporadically and were not collected from January 7, 1975 until April 14, 1975. On May 1, 1975, *Physa* was collected in the control plot but did not appear in the treated plot collections until August 17, 1975. Approximately 50% of the snail population was killed by the methoprene treatments in both habitats of the treated plot when compared to the untreated plot.

No *Palaemonetes paludosus* (Gibbs) were collected from the control plot until January 7, 1975, and they did not appear in the treated plot until July 1, 1975. Prior to this time, four methoprene treatments had been applied. These data indicate that although methoprene could have been responsible for the reduction in the treated population, environmental factors seemed to play a greater role.

Miura and Takahashi (1973, 1974) reported "no visible changes" in clam (*Dulimnadia* sp.) and seed (*Cypricerus* sp.) shrimp treated with methoprene in irrigated pastures. They reported 3.3% mortality to *Eulimnadia* sp. treated with methoprene in cage tests lasting 24 hr. In a 2nd test with the same shrimp they reported no mortality in cage tests after 72 hr exposure. They reported no mortality to *Cypricerus* sp. in cage tests after a 24 hr exposure.

Highly significant ($P < 0.01$) increases occurred in treated populations of *Trichocorixa louisianae* Jaczewaski immatures, *Psychoda* sp. immatures and *Procambarus clarki* (Girard) and *Cambarellus* sp. (mixed ages) when compared to the control populations (Table 2). In addition, significantly ($P < 0.05$) more *Liodessus affinis* (Say) adults were collected from the treated plots.

The population numbers of immature *Trichocorixa louisianae* increased in the vegetative habitat of the treated plot while no change in population density of the open water habitat was observed. A significant ($P < 0.05$) negative correlation was shown between immature *louisianae* and coenagrionid naiads. Significantly ($P < 0.05$) more coenagrionid naiads were collected from the vegetative habitat than the open water habitat in the untreated plot. The methoprene treatment caused a 47% reduction of coenagrionid naiads in the vegetative habitat of the treated plot which probably caused the 70% increase in the number of immature *T. louisianae* collected from this habitat.

The increase in *Psychoda* sp. larvae was probably due to the reduction of predators by treatment with methoprene. There was a significant ($P < 0.01$) negative correlation between psychodid larvae and coenagrionid naiads which were significantly reduced ($P < 0.01$) by the methoprene treatments. According to Smith and Pritchard (1973), the naiads of all Odonata are predaceous and the coenagrionids belong to a group of highly efficient predators.

The methoprene treatments caused a 44% reduction in the number of coenagrionid naiads in both habitats of the treated plot when compared to the untreated plot. This reduction in predator pressure (average of 0.87/0.09 m^2) probably caused the collection of significantly larger numbers of *Psychoda* sp. larvae in the methoprene treated plot as compared to the untreated plot where the coenagrionid naiad population averaged 1.56/0.09 m^2.

Table 2. Populations of aquatic organisms significantly increased after exposure to 6 applications methoprene (28 gm AI/ha/application over an 18 month period in a Louisiana intermediate marsh.)

	$\bar{X}$ No. Organisms Per $0.09m^2$					
	Control			Treated		
Species	Plants	vs Open	$\bar{X}$	Plants	vs Open	$\bar{X}$
Trichocorixa louisianae I	5.56	13.76[b]	9.57	9.43	13.74[b]	11.59[b]
Psychoda sp. I	0.10[b]	0.03	0.01	0.04	0.40[b]	0.22[b]
Procambarus clarki and *Cambarellus* sp. A&I	1.17[b]	0.59	0.88	1.79[b]	1.22	1.50[b]
Liodessus affinis A	0.10[b]	0.01	0.05	0.20[b]	0.07	0.13[c]

[a] A = adult, I = immature.
[b] Significantly different ($P < 0.01$).
[c] Significantly different ($P < 0.05$).

During the 18 month study, significantly ($P < 0.01$) more adult and immature *Procambarus clarki* and *Cambarellus* sp. were collected from the vegetative habitats of the treated and untreated plots (Table 2). However, after the methoprene treatments, there was over a 100% increase in the number of crawfish collected in the open water habitat of the treated plot when compared to the untreated plot. Likewise, significantly ($P < 0.01$) more adult *Liodessus affinis* were collected in the vegetative habitats of both the treated and untreated plots. The number of adult predaceous diving beetles increased in the vegetative and open water habitats of the treated plot as a result of the methoprene treatments. This increase in the crawfish and adult predaceous diving beetle populations was probably due to the control of predator species, but no negative correlations were obtained to substantiate this relationship.

No statistically significant ($P > 0.05$) increase or reduction was detected between the treated and untreated populations of adult *Lixellus* sp., *Hydrovatus cuspidatus* (Kunze), *Berosus exiguus* (Say), *Suphisellus* spp.; adult and larval *Lissorhoptrus* spp., *Laccophilus proximus* Say, *Enochrus blatchleyi* (Fall), *Tropisternus lateralis*; adult and nymphal *Buenoa* spp., *Belostoma testaceum* (Leidy), *Mesovelia mulsanti* Jaczewski; nymphal *Caenis* sp., *Pachydiplax* sp., *Trichocorixa louisianae* Jaczewski; larval *Liodessus affinis* (Say), *Anopheles* sp., *Culex salinarius* Coquillett, *Eulalia* sp., Dolichopodidae, *Lispe* sp.; and mixed ages of *Gambusia affinis* (Baird and Girard) and *Heterandria formosa* Agassiz.

Six applications of methoprene (28 gm AI/ha/application) over an 18-month period significantly reduced the population numbers of 14 aquatic organisms inhabitating this intermediate marsh. However, none of the affected organisms was eliminated as a result of these pesticide applications while the drought that occurred from July to late August, 1974, affected all aquatic organisms in thousands of hectares of marshland. Populations of 5 aquatic organisms were increased as a result of the methoprene applications and no significant ($P > 0.05$) difference was detected in the population numbers of 28 aquatic organisms exposed to the treatments. The authors concluded from these data that: 1) although methoprene applied to specific mosquito breeding sites in the marsh would cause reductions in the population numbers of certain aquatic organisms, no organisms would be completely eliminated from the ecosystem, 2) the control of predator species would cause corresponding increases in certain aquatic prey populations, and 3) repopulation of the treated area would occur from adjacent untreated marsh as was shown by the recovery of the aquatic organism populations following the drought of 1974.

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SUBSTRATE MOISTURE EFFECTS UPON OVIPOSITION IN *AEDES VEXANS*

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ABSTRACT. A laboratory experiment using 12 cohorts of *Aedes vexans* was used to determine the preferred substrate moisture for ovipositing females. Two substrates were used: cheesecloth and sand. On both substrates the extremes (0% and 100% saturation content) were avoided. On cheesecloth, the preferred moisture level was 20% with a decline in preference as the moisture level increased. On sand, there was no clear optimal moisture. Sand moistures between 20 and 80% saturation content were equally preferred. Speculation for a difference in the moisture preference regimes on 2 substrates is included.

INTRODUCTION

Aedes vexans is one of the most widespread pest mosquitoes in the world. Recently in an *AMCA Newsletter*, the regional directors were polled as to which mosquito species constituted the most serious pest in their areas. *Ae. vexans* was given the dubious honor without exception. Within the Midwest, control of this species alone would virtually constitute satisfactory mosquito control for many communities (Siverly 1972).

Since *Aedes* oviposit on the substrate surrounding a body of water, they rely on a rise in water level to hatch their eggs. Several investigators have been able to identify certain necessary requirements for field oviposition sites. Clements (1963) cited substrate moisture as the most fundamental physical attractant. Eggs begin to appear in sites as the water recedes following inundation in the spring and summer. Eggs are laid on soil that is moist but not water-logged, in a zone above the water table (Horsfall et. al 1973). Knight and Baker (1962), using *Ae. taeniorhynchus* and *Ae. sollicitans*, were the first to quantify the amount of substrate moisture in preferred oviposition sites. Peak attractiveness was in a range centered on 85% saturation content. By using similar methods this study quantifies the substrate mois-

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