EFFECTS OF THE GROWTH REGULATOR METHOPRENE ON *CULEX TARSALIS* AND NON-TARGET ORGANISMS IN CALIFORNIA RICE FIELDS

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ABSTRACT. Two formulations of the insect growth regulator methoprene were applied aerially (0.1 lb active ingredient/acre) to rice fields in the Sacramento Valley of California. The level of control of *Culex tarsalis* was assessed in emergence cages established before and after spraying in these and control fields. In cages established immediately after spraying, treated fields had about one half the rate of *Cx. tarsalis* emergence as did control fields. The level of control diminished to ca. 10 to 30% reduction by the 4th day after spraying, and by the 7th day emergence rates in treated fields were not significantly different from those exhibited before treatment began. The abundance of various non-target organisms was simultaneously assessed in treated and control fields. None of the organisms examined exhibited population fluctuations which could be statistically attributed to the methoprene applications.

INTRODUCTION

In the Sacramento Valley of California typically over 400,000 acres of land are devoted annually to rice production. These fields are usually flooded in early May. As the rice emerges, populations of *Culex tarsalis* develop in the warm water and usually reach a peak in mid-June. As the season progresses the rice shades and cools the water. In July and August *Anopheles freeborni* predominates (Bailey and Gieke 1968). These two mosquitoes are not only serious pests but also pose a threat to public health as vectors of western equine and St. Louis encephalitis and malaria.

Partial chemical control may be achieved with parathion or chlorpyrifos usually sprayed by airplane at 0.1 lb per acre (Womeldorf and Whitesell 1972). However, because of the rapid appearance of resistant mosquito strains, the chemical residue problems in rice, and the effect on non-target organisms, other methods of control have been sought. The mosquito fish, *Gambusia affinis* has been stocked in many rice fields with at least partial success (Hoy and Reed 1970, Hoy et al. 1971, 1972). However, it is difficult for mosquito abatement districts to regularly acquire adequate numbers of these fish for large scale use. Further, in some instances *Gambusia* have been unsuccessful in reducing mosquito numbers (Ahmed et al. 1970).

A recent addition to the chemical mode of control is the use of insect growth regulators (IGR). In a number of field situations these compounds have been effective mosquito control agents (Schaefer and Wilder 1972, 1973, Mulla et al. 1974, Steelman et al. 1975). The present study was conducted to determine the efficacy of one such compound, methoprene (Altosid®) in controlling *Cx. tarsalis* in northern California rice fields and to assess the effect on associated non-target organisms.

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MATERIALS AND METHODS

Low larval mosquito densities and extreme variation in numbers from field to field often make quantitative sampling difficult in California rice fields. To circumvent this problem, aquatic sweep nets were used instead of dippers to determine the relative density of mosquitoes in a number of rice fields in Sutter and Yuba Counties. Two adjacent fields which yielded large numbers of *Cx. tarsalis* were selected for study. The following 2 formulations of methoprene (isopropyl-11

-methoxy-3, 7, 11-trimethyl-dodeca-2,4 dienoate) were tested at 0.1 lb active ingredient per acre: 1) the standard micro-encapsulated product and 2) a 10F charcoal formulation thought to be more stable. Three adjacent checks were selected from 1 field as replicates for each of the 2 treatments, and 3 checks from the neighboring fields served as controls. (A check is simply the term used to describe a section of a rice field between two levees.)

Aerial spraying was conducted on July 5, 1974, approximately 55 days after planting. The rice canopy at this time was about 33 cm above the water surface, and water depth was approximately 18 cm. Maximum daily water temperatures exceeded 32°C in all checks. Both methoprene formulations were applied by a Piper Cub flying at 40mph at about 30ft elevation. The spray system nozzle was No. 88010 and was set for 55 psi yielding a 50ft swath. One gal of the standard formulation was diluted with 9 gals of water and applied at a liquid volume of 1 gal/acre. Two qt of the charcoal formulation were mixed with 9 gal of water and also applied at a liquid volume of 1 gal/acre.

One week prior to spraying, gallon-sized plastic emergence cages with organdy screened windows and bottoms and floats near the top were placed about 6 to a check near the tail end. Thirty 4th-instar *Cx. tarsalis* larvae obtained by sweeping each check were placed inside each bucket which was then capped with an organdy screen top. On a daily basis, adults which emerged in each of these cages were counted and released. New emergence cages were established immediately after spraying, 4 days after spraying, and again 7 days after spraying. Each bucket was scored daily until all mosquitoes had either emerged or died.

A sample of the total aquatic fauna was obtained from each plot twice weekly by taking 30 uniform sweeps with a fine-mesh aquatic sweep net. This sampling began 1 wk prior to treatment and ended 3 wk after treatment. The contents were returned to the laboratory for counting and identification. Statistical analyses on these data were performed on a Burroughs B6700 computer at the U.C. Davis computer center.

RESULTS AND DISCUSSION

The level of *Cx. tarsalis* control achieved by the 2 formulations of methoprene is shown in Table 1. Analysis of variance revealed no significant differences in percent emergence between the 2 groups of cages established in treated and control plots 7 days prior to treatment. Emergence cages established immediately after treatment indicated ca. 50% reduction of *Cx. tarsalis* had been achieved ($P<.001$) under both methoprene formulations compared to untreated and pre-treatment levels. This difference in emergence was still significant by the 4th day, but had diminished to about 10% reduction with the standard methoprene formulation and about 30% reduction with the 10F charcoal formulation. By the 7th day emergence was not significantly different from pre-treatment levels with either formulation. The average daily rate of emergence in cages established on day 0 is depicted in Fig. 1. In both treated and untreated plots emergence asymptotically approached completion after 10 days.

Aquatic sweep net samples yielded numbers of 12 of the aquatic organisms which were sufficient for statistical comparisons: gastropods, ostrocods, spiders, dragonflies, giant water bugs (*Belastoma*), midges (Chironomidae), *Laccophillus decipiens* (Coleoptera:Dytiscidae), *Tropisternus lateralis* (Coleoptera:Hydrophilidae), *Cx. tarsalis*, *Anopheles freeborni*, Ceratopogonidae and Stratiomyiidae. The effect of methoprene treatments on these organisms was evaluated using 2 different analysis of variance designs.

First, the sampling dates were divided into the following 3 groups: 1) those samples taken prior to treatment (days −7 and −2); 2) the samples taken on days 3, 6, and 9 and; 3) the samples taken on days

13, 18, and 23. For each group of dates and for each of the 12 organisms a two-way analysis of variance was performed, comparing the effect of the 2 independent variables (sampling time within a data group and treatment) on the single dependent variable, the relative numbers of the aquatic organism. This yielded 36 analyses (3 date groups times 12 organisms). The results are displayed in Table 2. A downward pointing arrow indicates a significant reduction in numbers due to treatment (left side) at that time interval or a decrease in numbers through time (right side) during that time interval. An upward pointing arrow indicates an upward trend.

Prior to treatment, the different plots were statistically homogeneous and fairly stable in their species composition since no significant F-values were obtained. During the 1st week after treatment dragonflies, *Belastoma, Tropisternus,* and Stratiomyiidae exhibited smaller numbers in both groups of treated plots compared to control checks. With the exception of

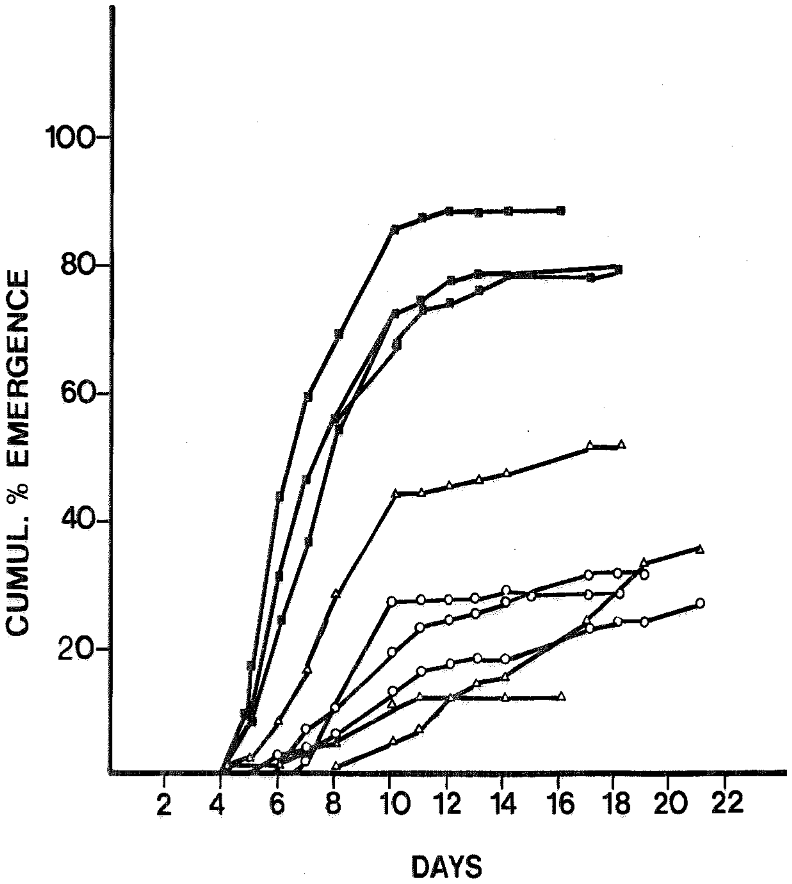

Fig. 1. The average cumulative percent emergence through time for emergence cages established immediately after spraying in each check of treated and untreated fields. □ = control checks; 0 = standard methoprene formulation; Δ = 10F charcoal formulation.

Table 1. The effect of methoprene treatment on the percent emergence of fourth instar *Culex tarsalis* in field emergence cages.

	Methoprene			Charcoal Methoprene			Control		
Days after spraying	Mean % emergence	± 2S.E.	N	Mean % emergence	± 2S.E.	N	Mean % emergence	± 2S.E.	N
−7	82	8.5	15	73	6.3	17	66	4.8	18
0	29	9.1	18	32	13.1	17	80	7.9	18
4	64	7.4	16	42	10.9	16	74	8.7	8
7	76	7.5	14	69	13.1	15	—	—	—

Tropisternus this was true for the period 13 to 23 days after spraying. In this latter period ostracods, chironomids and *An. freeborni* showed significant increases through time. Since the density of these animals was not significantly affected by treatment this resurgence presumably represents a natural seasonal growth.

In every instance where significant effects due to treatment were evident the actual magnitude of the difference in numbers between pre- and post-treatment levels was low. Furthermore, in most cases, the same trend in average numbers between control and treated plots was evident (but not statistically significant) prior to treatment. Recalling that both groups of treated checks were in one rice field but control checks were in an adjacent field with a separate water flow, it seems possible that the natural succession of organisms was slightly different in these 2 fields even prior to treatment. We suspect that this trend became magnified through time, without any impetus ascribable to the methoprene application. Concurrent studies on the natural succession of organisms through rice field habitats have emphasized the extreme variability from field to field.

Next, a multivariable one-way ANOVA design was tested comparing the effect of treatment on all 12 species simultaneously, for each sampling date. This yields 8 tests. No significant differences were found among the 3 groups of plots before or after spraying except for day 23. This reinforces our conclusions drawn from the single variables ANOVAS. That is, methoprene had little direct effect on the numbers of these aquatic organisms in this rice field habitat.

At 0.1 lb per acre, methoprene achieves only partial control of *Cx. tarsalis* and this lasts at most 3 or 4 days. At this dosage, the cost of application (approximately $1.00 per acre) is substantially greater than that which mosquito abatement districts in the Sacramento Valley are presently paying in their rice field control efforts, thus precluding any higher dosage rates. A number of factors contribute to the lack of success in these trials, certainly not the least of which is the lower toxicity of methoprene to *Cx.* mosquitoes compared to *Aedes* species (Schaefer and Wilder 1972, 1973). Environmental factors contributing to a reduction in control are the relatively high water temperatures and light exposure in the rice habitat, the rapid flow of water through the system, and the asynchronous age structure of the mosquito population (see e.g., Schaefer and Dupras 1973). In southern Louisiana where rice is cultivated without continuous water flow and where fields are periodically drained and reflooded, *Psorophora* mosquitoes are pestiferous. In this case the populations' age structure is synchronized and methoprene as well as other IGR compounds have been found to yield satisfactory control (Steelman et al. 1975).

The other major mosquito in California rice fields is *Anopheles freeborni*. The relatively low larval densities of this species have hampered attempts to determine its

Table 2. The average numbers of various rice field aquatic animals in three replicate plots. Samples were obtained by taking 30 sweeps through each plot with an aquatic net.

	Pretreatment					3 to 9 days post-treatment					13 to 25 days post-treatment				
Organism	T_1	T_2	C	sig.	sig.[1]	T_1	T_2	C	sig.	sig.	T_1	T_2	C	sig.	sig.
Gastropoda	.3	.8	1.2			1.8	.7	1.9			0	.6	2.6		
Ostacoda	10.5	16.7	0			28.0	20.9	5.9			42.0	67.8	.9		↑
Spiders	6.5	16.3	5.3			6.3	5.8	4.6			7.2	6.0	3.3		
Dragonfly nymphs	.3	1.8	1.3			.8	.7	4.6	↓		1.7	.2	8.2	↓	
Belastoma	0	0	.2			.2	0	1.2	↓		.3	.3	2.3	↓	
Chironomidae	0	0	0			0	.1	0			3.8	34.4	4.1		↑
Laccophillus decipiens[2]	3.2	19.0	6.8			10.7	18.3	10.1			16.1	17.3	5.2		
Tropisternus lateralis[2]	4.3	20.8	11.5			11.7	6.8	18.3	↓		13.8	13.9	10.0		
Culex tarsalis	7.7	18.0	30.0			5.9	9.6	20.1	↓		1.6	2.1	7.4	↓	
Anopheles freeborni	.5	.8	.2			2.9	2.2	.9			4.6	1.9	1.1		↑
Ceratopogonidae	0	0	6.7			.8	0	33.0	↓		.1	.1	15.1	↓	
Stratiomyiidae	0	1.7	1.7			0	0	5.3	↓		.3	.3	7.2	↓	

[1] An arrow on the left side of the column represents a significant difference ($P < .05$) between both types of treated plots and the control fields using an analysis of variance (see text for exact design). If the arrow is downward, then treated plots had significantly lower densities than controls. An arrow on the right side of the column expresses a significant temporal trend in relative density. An upward arrow indicates the organism significantly increased during that period and a downward arrow means the converse. The absence of an arrow indicates no significant F-value. T_1 represents methoprene treated fields; T_2 charcoal methoprene treated fields; C, control fields.

[2] Includes adults and immature stages.

susceptibility to IGR compounds under field conditions. One preliminary study (Dunn, Case, and Washino 1974) indicated *An. freeborni* may be more susceptible to methoprene, but as in the present study any effects had disappeared by the 3rd day after treatment.

The unique mode of action of insect growth regulators precludes the usual short term approach to evaluating nontarget effects. The assessment of the relative density of various aquatic organisms in treated and untreated plots, as in this study, does not allow us to draw any conclusions on the ultimate causes of these differences. For example, a decline in numbers of one species due to the direct action of methoprene may disrupt the food web causing indirect increases or decreases in other species which are unaffected by the compound.

Although at times statistically significant, the effects of methoprene on the numbers of nontarget organisms were of relatively minor magnitude. The wide array of taxonomic groups affected and the delay of at least a week before these effects became evident indicate that these animals may have experienced such indirect effects through perturbations in the food web. An alternative hypothesis is the methoprene damaged early and more sensitive life stages such as the eggs of these organisms. In this regard Dunn (unpublished) has found that a number of IGR compounds including methoprene are extremely detrimental to corixid eggs. Less extreme but still significant effects were observed on the pupation behavior of the giant water beetle *Hydrophilus triangularis* (Dunn et al. 1974).

Because of the continuous flow of water through our rice plots there is a continual immigration of new individuals into the study area. Likewise there is probably significant aerial immigration of adult insects into our plots from widespread untreated areas. This, to some extent, will mask any local mortality which methoprene may have induced. The relative tolerance of the nontarget organisms in this study in no way guarantees that more widespread use of this compound would not have more damaging effects.

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