EFFICACY OF NEW INSECT GROWTH REGULATORS AGAINST MOSQUITO LARVAE IN DAIRY WASTEWATER LAGOONS[1]

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ABSTRACT. Four new insect growth regulators (IGRs) and a slow-release formulation of a currently-used IGR were evaluated for the control of *Culex peus* and *Cx. quinquefasciatus* in dairy wastewater lagoons. The IGR AC-291898 (CME 13406) proved highly efficacious, producing 100% control for one week and about 98% control for two weeks at the rate of 0.05 lb AI/acre (0.056 kg/ha). The IGR XRD-473 produced similar results at this rate. The effective rate of these two compounds seems to be in the range of 0.05 to 0.1 lb AI/acre (0.056-0.11 kg/ha). A granular formulation of S-31183 (0.5 G) applied at 0.05 lb AI/acre (0.056 kg/ha) yielded mediocre reduction whereas fenoxycarb EC 1 at up to 0.25 lb AI/acre (0.28 kg/ha) and methoprene 4% slow release pellets at up to 1.0 lb AI/acre (1.12 kg/ha) produced little or no control of *Culex* in the dairy wastewater lagoons. These compounds need to be applied at higher rates or suitable formulations will have to be developed to achieve satisfactory control.

INTRODUCTION

Culex quinquefasciatus Say and *Cx. peux* Speiser breed heavily in animal wastewater lagoons (Axtell et al. 1980, Mulla et al. 1988b, O'Meara and Evans 1983). Their control in these highly eutrophic habitats is extremely difficult. For example, various formulations of *Bacillus thuringiensis* H-14, failed to produce adequate control of *Cx. quinquefasciatus* larvae at the practical larvicidal rates when applied in polluted water habitats (Mulla et al. 1982, Rathburn et al. 1984). Similar trends of lower activity were reported for *B. sphaericus* 2362, some organophosphorus larvicides and insect growth regulators (IGRs). Flit MLO (7 gal/acre) and temephos (0.5 lb AI/acre) produced satisfactory control for 3–4 days only, whereas methoprene and malathion failed to render adequate control at the rates of 0.4 and 1.0 lb AI/acre, respectively (Axtell et al. 1980). *Bacillus sphaericus* 2362 formulations, a primary powder (ABG-6184) and a liquid formulation (BSP-2), were highly active against *Cx. tarsalis* Coquillett in clear water at the rates of 0.05 and 0.5 lb/acre, respectively (Mulla et al. 1986a). However, these rates yielded poor results against *Cx. peus* in dairy wastewater lagoons. A 5–10 fold increase in these rates was required to produce significant initial and persistent control (Mulla et al. 1988b).

According to published information, generally higher rates of mosquito larvicides are required to obtain adequate larval control in polluted animal wastewater lagoons. Factors affecting activity and longevity of larvicides under these conditions are the daily dilution of treated water in the lagoons, the rapid degradation of the toxicants, and their adsorption onto organic matter, as reported by Schaefer et al. (1987).

Recently, several IGRs were reported to exhibit high levels of biological activity against several species of stagnant and floodwater mosquitos, including *Aedes nigromaculis* (Ludlow), *Ae. melanimon* Dyar, *Psorophora columbiae* (Dyar and Knab), *Cx. tarsalis* Coquillett and others (Bassi et al. 1987, Estrada and Mulla 1986; Mulla et al. 1985, 1986b). These highly-active IGRs include various formulations of fenoxycarb and an experimental IGR, S-31183. Both fenoxycarb and S-31183 were evaluated and found to be highly active against floodwater and stagnant water mosquitoes but their efficacy in polluted water situations has yet to be determined. This study was conducted to evaluate these and other new IGRs against *Culex* mosquito larvae in dairy wastewater lagoons.

MATERIALS AND METHODS

Insect growth regulators evaluated were fenoxycarb EC 1 [ethyl(-p-phenoxyphenoxy) ethylcarbamate] (Maag Agrochemicals, Vero Beach, FL), S-31183 0.5 G [1-(4′-phenoxyphenoxy)-2-(2′-pyridyloxy)-propanel] (Sumitomo Chemical Co., Osaka, Japan), AC-291898 or CME 13406 10% EC [1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl)-urea] (American Cyanamid Co., Princeton, NJ), XRD-473 5% EC [N-[(((3,5-dichloro-4-(1,1,2,2-tetrafluoroethyoxy)-phenyl)amino) carbonyl)-2,6-difluorobenzamide] (Dow Chemical Co., Midland, MI), and methoprene 4% pellets (Zoecon Corp. Dallas, TX).

Tests were conducted in dairy wastewater lagoons at Lakerkirk and Kasbergen dairies in Riverside and San Bernardino counties, California. Mosquito larval populations at both locations consisted mostly of *Cx. peus* (90–95%) but

[1] These studies were conducted in cooperation with Northwest Mosquito Abatement District, Riverside and West Valley Vector Control District, Chino, California. They were supported by State of California Special Fund for Mosquito Research.

Cx. quinquefasciatus larvae were also present in small numbers (5–10%). Plots at Kasbergen dairy were 0.25–0.3 acre (1,010-1,350 m^2) and square in shape. At Lakerkirk, plots were rectangular and consisted of 0.15–0.25 acre (675–1,010 m^2).

The S-31183 (0.5% G) granules and methoprene 4% pellets were broadcasted by hand along the sides and toward the middle of the lagoons. To ensure even coverage, the required amount for each rate of application was divided into four portions, and one portion was applied from each side of the square plots. In the rectangular plots, the required amount was divided into two equal portions, utilizing one portion per side. The required amount of each of the emulsifiable concentrates was mixed with tap water and applied with a hand sprayer fitted with a 8006 Teejet nozzle at the rate of 8 gal of aqueous spray per acre. In all tests, one lagoon was used per application rate and an adjacent lagoon of similar size was left untreated as check.

To determine activity and longevity of the IGRs against the larval population, two methods of evaluation were utilized as described by Mulla et al. (1986b): i.e.,

1. Standard dipper method. To measure larval mortality, 20 dips per plot were taken prior to treatment and at 2, 7 and 14 days after treatment. Percent reduction calculation was based on the number of 3rd- and 4th-instar larvae in posttreatment vs. pretreatment counts.

2. Larval isolate method. To determine residual activity, water and 4th-instar larvae of *Cx. peus* were collected from treated and check plots and were transported to the laboratory in 2 gal (8 liter) size buckets (Rubbermaid Inc., Wooster, OH) lined with double plastic bags. On each sampling interval, 200 ml of each treated and check water and 20 associated larvae were transferred into 10-oz disposable salad foam bowls in triplicate. Test organisms were maintained at 24 ± 1°C. Mortality readings were taken daily until all organisms either died or reached the adult stage normally. When complete larval mortality occurred in one of the test plots, laboratory larvae of *Cx. quinquefasciatus* were utilized in field-collected water. This method also measures delayed mortality in the larval, pupal and adult stages.

RESULTS AND DISCUSSION

The new IGR AC-291898 (10% EC) induced 56 and 98% mortality in the larval population of *Cx. peus* 2 and 7 days after treatment, respectively. No reduction in the larval population was obtained 14 days after treatment at the rate of 0.025 lb AI/acre (0.028 kg/ha) (Table 1). At the higher rate of 0.05 lb AI/acre (0.056 kg/ha), excellent larval control was achieved for 14 days.

The IGR XRD-473 (5% EC) yielded little or no larval mortality at the low rate of 0.025 lb AI/acre (0.028 kg/ha). The higher rate of 0.05 lb AI/acre (0.056 kg/ha) induced complete mortality for 7 days. However, the pond dried and percent larval mortality could not be ascertained after this period.

In the laboratory, larval isolates, treated with AC-291898, yielded 100, 69 and 68% inhibition of adult emergence (EI) at 2, 7 and 14 days after treatment at the low rate of 0.025 lb AI/acre (0.028 kg/ha) (Table 2). Complete inhibition of adult emergence occurred at the higher rate of 0.05 lb AI/acre (0.056 kg/ha) at 2 and 7 days posttreatment. At 14 days posttreatment, this rate gave 39% EI. This additional inhibition of emergence is over and above the high level of larval reduction (Table 1). The IGR XRD-473 produced low larval mortality at the low rate of 0.025 lb AI/acre (0.028 kg/ha) (Table 1). Most mortality occurred in the larval, pupal and adult

Table 1. Efficacy of IGRs against *Culex peus* larvae in dairy wastewater lagoons as assessed by the dipping technique

Rate lb AI/acre	Plot size (acre)	Larvae/ dip pre-treatment	(%) reduction in larvae after treatment (days)		
			2	7	14
		AC-291898 (10% EC)			
0.025	Lakerkirk (0.25)	57	56	98	0[a]
Check	Lakerkirk	17	0	0	0
0.05	Kasbergen (0.3)	116	100	100	95
Check	Kasbergen	24	0	0	0
		XRD-473 (5% EC)			
0.025	Lakerkirk (0.15)	48	39	0	0
Check	Lakerkirk	26	0	0	0
0.05	Kasbergen (0.3)	46	72	100	dry
Check	Kasbergen	7	0	0	0

[a] 75% of water surface was covered with dead adults (200/dip).

Table 2. Efficacy of IGRs against *Culex peus* larvae in dairy wastewater lagoons as assessed by the larval isolate method[a]

Rate lb AI/acre	(%) Mortality in larval isolates after treatment (days)											
	2				7				14			
	L	P	A	(%EI)	L	P	A	(%EI)	L	P	A	(%EI)
AC-291898 (10%) EC												
0.025	81	19	0	100	3	8	58	69	2	7	59	68
0.050	43	57	0	100	0	90	10	100	1	2	36	39
Check	0	0	0	0	0	1	0	1	12	2	0	14
XRD-473 (5%) EC												
0.025	31	18	29	78	0	0	5	5	—	—	—	—
0.050[b]	100	0	0	100	100	0	0	100	1	1	65	67
Check	0	0	0	0	1	2	0	3	8	3	0	11

[a] Location and plot size are the same as shown in Table 1.
[b] Larvae were eliminated in test plot. Laboratory larvae of *Cx. quinquefasciatus* were utilized in field-collected water.

stages at this low rate. Complete inhibition of adult emergence was obtained 2 and 7 days after treatment at the high rate of 0.05 lb AI/acre (0.056 kg/ha) (Table 2). At this high rate, mortality occurred in the larval stage.

AC-291898, on the other hand, induced mortality in the larval and pupal stages 2 days posttreatment, and in pupal and adult stages 7 and 14 days posttreatment at both rates (Table 2). About 75% of treated water surface with AC-291898 was covered with dead adults 14 days after treatment (Table 1). Upon examination, dead adults were almost completely emerged but hindlegs remained attached to the pupal skins. As the concentration of this compound decreases, survivorship of larvae increases and delayed mortality then insues in the pupal and adult stages.

The population of *Cx. peus* larvae treated with fenoxycarb (EC 1), S-31183 (0.5% G) and methoprene 4% pellets remained high, and no reduction in the larval population was observed 2 and 7 days after treatment (data omitted). Most surviving larvae isolated 2 and 7 days after treatment with fenoxycarb at 0.1 and 0.25 lb AI/acre (0.112–0.28 kg/ha) and methoprene 4% pellets at 1.0 lb AI/acre (1.12 kg/ha) reached the adult stage normally. However, S-31183 (0.5% G) caused 63 and 66% EI at the rates of 0.025 and 0.05 lb AI/acre (0.028 and 0.056 kg/ha), respectively, 2 days after treatment, and poor results were obtained 7 days after treatment (Table 3).

As the data indicate, methoprene 4% pellets and S-31183 (0.5% G) are unsuitable for mosquito control in dairy wastewater lagoons. It is possible that these pellets and granular formulations sink rapidly into the bottom of the pond and toxicants released were adsorbed to organic matter at the bottom, thus limiting their activity against mosquito larvae in polluted water. In recent studies, methoprene 4% pellets yielded excellent control of *Cx. tarsalis* and *Cx. peus* larvae in clear water ponds at rates 0.1–0.25 lb AI/acre (0.112–0.28 kg/ha) (Mulla et al. 1988a). S-31183 (0.5% G) also produced excellent control of these two species and the floodwater mosquitoes *Ae. nigromaculis*, *Ae. melanimon* and *Ps. columbiae* at the low rate of 0.005 lb AI/acre (5.6 g/ha) (Mulla et al. 1986b, 1988a). Additional studies are planned to determine initial efficacy and longevity of methoprene 4% pellets as pre- and posthatch treatments against floodwater mosquitoes in irrigated fields.

Table 3. Efficacy of IGRs against *Culex peus* larvae in dairy wastewater lagoons as assessed by larval isolate methods[a]

Rate lb AI/acre	(%) Mortality in larval isolates after treatment (days)							
	2				7			
	L	P	A	(%EI)	L	P	A	(%EI)
Fenoxycarb (EC 1)								
0.100	0	0	0	0	0	3	0	3
0.250	0	11	6	17	0	15	8	23
Check	0	10	3	13	0	5	3	8
S-31183 0.5 G								
0.025	0	43	20	63	0	20	6	26
0.050	0	55	11	66	0	24	5	29
Check	0	6	1	7	0	1	1	2
Methoprene (4%) pellets								
0.500	0	1	0	1	0	5	6	11
1.000	0	8	10	18	8	0	8	16
Check	0	0	0	0	0	0	0	0

[a] Tests were conducted in 0.25–0.3 acre (1,010–1,350 m^2) plots at Kasbergen dairy.

Fenoxycarb (EC1) and S-31183 (0.5% G) produced poor results against *Cx. peus* larvae in dairy wastewater lagoons at the low rates applied. These materials should be evaluated at

higher rates to determine their optimum rates of application in polluted water. In conclusion, XRD-473 5% EC and AC-291898 10% EC produced excellent results against *Cx. peus* larvae in dairy wastewater lagoons and could be used effectively at rates of 0.05–0.1 lb AI/acre (0.056–0.112 kg/ha). There is a great need for further development and labeling of these compounds for mosquito control in wastewater lagoons.

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