EFFICACY OF A JUVENILE HORMONE MIMIC, PYRIPROXYFEN (S-31183), FOR MOSQUITO CONTROL IN DAIRY WASTEWATER LAGOONS

F. S. MULLIGAN III AND C. H. SCHAEFER

Mosquito Control Research Laboratory, University of California, 9240 S. Riverbend Avenue, Parlier, CA 93648

ABSTRACT. Pyriproxyfen (S-31183) was applied to dairy wastewater lagoons, during 1988, at 0.1 kg (AI)/ha in single and multiple applications which resulted in control of *Culex* spp. larvae for periods of 7 to 68 days. Length of the control period appeared to be related to water quality, with greater residual efficacy in more polluted sources. The AI apparently adsorbed onto organic debris where efficacy remained high in the lagoon even after water was pumped from the lagoon and replenished with untreated wastewater. Alternating treatments with control agents having a different mode of action is suggested to avoid selection of insecticide resistance.

INTRODUCTION

Dairy wastewater lagoons are used to collect wash water and animal fecal material from holding pens and milk barns. The high degree of organic matter in the water is attractive to ovipositing mosquitoes and conducive to immature development. The abundance of *Culex quinquefasciatus* Say breeding in such lagoons is related to the level of organic pollution. Population density is also enhanced by increased vegetation and floating debris (Rutz and Axtell 1978).

Animal wastewater lagoons represent a habitat where the potential for insecticide resistance is very high, due to the large numbers of *Cx. quinquefasciatus* which breed in the lagoons and the frequent insecticide treatments necessary for control (usually every 7–10 days over a substantial portion of the calendar year). Past experience has proven that the half-life of a promising new chemical agent may be limited to a few seasons if consistent selection pressure is placed on the *Cx. quinquefasciatus* populations in animal wastewater lagoons. For example, Schaefer and Dupras (1970) demonstrated that chlorpyrifos offered rather long-term control of mosquito larvae in polluted water sources. Mosquito abatement districts in the San Joaquin Valley of California began regular treatments of animal wastewater lagoons with chlorpyrifos and by 1974 control failures in these habitats were observed (Stewart 1975, Georghiou et al. 1975). Past experience shows that insecticide resistance can occur to any chemical agent if high numbers of organisms are exposed to selection pressure for an adequate period of time. Since new types of chemical agents are infrequently available, it is imperative that considerations of insecticide resistance be included in developing strategies for their use.

Pyriproxyfen, S-31183, 2-[1-methyl-2(4-phenoxyphenoxy) ethoxy] pyridine is unrelated structurally to natural insect juvenile hormones; but its biological activity is the same as for juvenile hormone type compounds (Schaefer et al. 1988). Previous studies with pyriproxyfen have shown it to be very active against *Aedes*, *Culex* and *Psorophora* species mosquitoes, both in the laboratory and in field studies at rates down to 0.0056 kg (AI)/ha (Estrada and Mulla 1986, Mulla et al. 1986, Schaefer et al. 1988, Mulla et al. 1989). While Mulla and Darwazeh (1988) reported low efficacy for pyriproxyfen in dairy wastewater lagoons at dosage rates of 0.028 and 0.056 kg (AI)/ha, Schaefer et al. (1988) reported complete control of mosquitoes in such lagoons for 2 months with a single pyriproxyfen application at 0.11 kg (AI)/ha. We endeavored to further examine the potential of pyriproxyfen as a mosquito control agent in this environment. Because of the high potential for development of resistance to pyriproxyfen during prolonged exposure of large populations of mosquitoes, we alternated applications of an entirely unrelated control agent (larvicidal oil) between multiple pyriproxyfen applications in our evaluation.

MATERIALS AND METHODS

Two lagoons, within the Consolidated Mosquito Abatement District in Fresno County, were selected to receive multiple pyriproxyfen applications. One lagoon was located at Nunes dairy and had an area of 0.032 ha. The other, larger lagoon, 0.34 ha, was at Qualls dairy. Water depth in both lagoons was estimated to be 2 m. Three dairy lagoons were selected for single applications of pyriproxyfen. One of these was at Jacobi dairy in Tulare County, CA, within the Delta Vector Control District, and it had an area of 0.36 ha and a water depth of ca 1.5 m. The remaining 2 lagoons were at St. Clair dairy in Fresno County: 1) a 0.069 ha × 2 m water depth, solids settling lagoon, and 2) a 0.14 ha × 1 m water depth, storage lagoon. The solids settling lagoon was designed to prevent floating and settleable solids from entering the storage lagoon.

The lagoons selected for treatment, during the summer and fall of 1988, had known histories of regular breeding of *Cx. quinquefasciatus*; and no comparable lagoons that could be left untreated as controls were available.

Pyriproxyfen was provided by McLaughlin Gormley King Company, Minneapolis, MN. The emulsifiable formulation, MGK Product F-2482, contained 10% AI weight/weight. The percent AI was verified with HPLC analysis by comparison to a standard prepared from technical material.

The initial application of pyriproxyfen at Nunes dairy was made with a hand can sprayer. All subsequent pyriproxyfen applications at Nunes dairy, as well as those at the other dairies were made with a sprayer mounted on a pickup truck, provided by the respective mosquito abatement or vector control district. Pyriproxyfen was applied as a water mix in sufficient volume to cover the entire surface area of the pond at a rate of ca 0.1 kg (AI)/ha. Three pyriproxyfen applications were made to the Nunes lagoon and to the Qualls lagoon, with one or more larvicide oil treatments interspersed between pyriproxyfen applications. The amount of Golden Bear GB-1356 larvicide oil (Witco Corp., Oildale, CA) applied at each oil treatment averaged 7.6 liters per lagoon at Nunes and 21 liters per lagoon at Qualls and were applied by mosquito abatement personnel with hand can sprayers.

The effect of pyriproxyfen upon the target mosquito populations was evaluated by collection of pupae from the wastewater lagoons. Duplicate samples of 50 pupae each were placed in ca 250 ml of tap water and held at 25±2°C in the laboratory until mortality and adult emergence were complete. At least twice on 2 days prior to treatment at each test site, untreated pupae were sampled to determine the natural mortality of the untreated mosquito population. Sampling was conducted at weekly intervals throughout the test duration. Along with the pupal collections at sampling dates, field observations on the mosquito populations were recorded. Water electrical conductivity and pH were observed and recorded at each lagoon throughout the study. Electroconductivity data were analyzed by the SAS general linear model procedure (SAS Institute 1985).

RESULTS

Results of the multiple pyriproxyfen applications to the Nunes and Qualls lagoons are summarized in Table 1. After the first applications, there was essentially 100% inhibition of adult mosquito emergence for 43 days at Nunes (with 96% inhibition at day 22); however, control lasted only 7 days at Qualls. As shown in Table 1, water was pumped from the Nunes lagoon on 2 occasions following the initial treatment, including a 1.1 m drop in water level 8 days after. The second Qualls application resulted in 90–100% inhibition through 14 days. Following the final application at Qualls, adequate control (97–99% inhibition) lasted for 22 days. Adult emergence was completely inhibited for 4 weeks, except for 99% inhibition on day 22, after the second Nunes application, which coincided with the third Qualls application. Once again water was pumped from the Nunes lagoon (3 times), which caused a water level drop of 1.7 m the day after treatment, leaving a depth of less than 0.3 m of water. The final application at Nunes resulted in 100% inhibition for 68 days, until December 7, when sampling was discontinued due to the onset of cold weather.

Applications of larvicide oil eliminated fourth instar larvae and pupae from the lagoons for 5–8 days. The durations of immature mosquito

Table 1. Percent inhibition of successful adult emergence from pupal samples collected from two dairy wastewater lagoons before and following 3 treatments with S-31183 at 0.1 kg (AI)/ha.

Nunes Dairy				Qualls Dairy			
Days after S-31183 treatments			Percent inhibition	Days after S-31183 treatments			Percent inhibition
−1			4	−21			7
0			2	−20			5
8			100[a]	−6			6
19			100	−2			5
22			96[a]	0			3
26			100[b]	5			91
34			—[b]	7			100
43			100	12			74
44			—[b]	15			35[a,b]
51			89	25			33
54			80	28	0		17
56	1		—[a]		4		100
	7		100		11		90
	16		100[a]		14		99[b]
	22		99		25		69
	28		100[a]		28	2	100
	35		64			7	97
	37		—[b]			16	98
	49	4	100			22	99
		11	100			28	80[a]
		19	100[a]			30	—[b]
		25	100			42	21
		32	100[a]				
		40	100				
		46	100				
		53	100				
		68	100				

[a] Wastewater was pumped from lagoon, reducing volume up to 80%.

[b] Alternate treatment with GB1356 larvicidal oil.

mortality effected by the oil treatments were short-lived, 1 or 2 days, before successful reinfestation occurred. Further applications of either oil or pyriproxyfen were made from 8 to 13 days after an oil application, with the resurgence of immatures and after collection and viability determination of pupae.

Single applications of pyriproxyfen were made to 3 dairy lagoons, with results shown in Table 2. Treatment of the St. Clair solids lagoon gave adequate control (97–99% inhibition) for 20 days, after which the water was pumped down to a level which could not be sampled. Applications to the St. Clair storage and the Jacobi lagoons yielded 100% inhibition for 11 and 17 days, respectively. High activity (96% inhibition) continued to day 24 at the Jacobi lagoon.

Mortality of the untreated mosquito populations from the wastewater lagoons was low. The highest natural mortality in a sample taken prior to treatment and held in the laboratory was 7%.

Culex quinquefasciatus was the only mosquito species identified from the Nunes, St. Clair and Jacobi pupal collections, by imaginal identification. However, out of a sample of 172 adults from the Qualls lagoon collections, *Cx. quinquefasciatus* accounted for 84%, with 13% *Cx. tarsalis* Coquillett and 6% *Cx. stigmatosoma* Dyar (=*peus*).

Electroconductivity of lagoon wastewater was stable for each lagoon, but varied by as much as 2× between lagoons. Measured in micromhos/cm, the mean conductivity values ± SD for each lagoon over the sampling period were 1,770 ± 100, 2,610 ± 230, 3,080 ± 170, 3,150 ± 500 and 3,420 ± 80, for Qualls, Nunes, St. Clair storage, St. Clair solids and Jacobi, respectively. Except for the St. Clair lagoons, the means differed significantly between lagoons (ANOVA $F = 64.33$, $P < 0.0001$, df= 4,31; Duncan's multiple range, critical range = 250 for 2 means). Wastewater pH measured between 7.1 and 7.8 in all lagoons.

DISCUSSION

Pyriproxyfen effectively inhibited emergence of adult mosquitoes in dairy wastewater lagoons for periods of from 1 week to 1 month and longer. At the Nunes dairy, three pyriproxyfen applications, alternating with larvicidal oil treatments, provided control for an entire season, from July through November. Residual control from each application persisted for about a month before an additional treatment was required. However, residual activity at Qualls was more short-lived and inconsistent: from 1 to 2 weeks of effective control. From 2 to 3 weeks of control was obtained in single applications to the 3 other dairy lagoons. Thus, if pyriproxyfen were incorporated into mosquito abatement operations at 0.1 kg (AI)/ha, effective residual control from 1 to 4 weeks could be expected.

It appears that the difference in length of residual activity is related to water quality. The Qualls lagoon appeared, by sight, to be less polluted; also its lower electroconductivity values indicated better water quality. Further, *Cx. tarsalis*, which generally prefers less polluted water, was collected only from the Qualls lagoon. Length of residual activity was also slightly reduced in the storage lagoon at the St. Clair dairy below that of the solids separating lagoon. Since the purpose of the solids lagoon was to decrease the amount of solids entering the storage lagoon, less organic matter was expected in the storage lagoon. Residual activity was enhanced in lagoons with a greater apparent organic component.

As in our earlier study (Schaefer et al. 1988), we speculate that pyriproxyfen adsorbed onto organic matter in the feeding zone and retained biological activity. The adsorbed material was available for ingestion by mosquito larvae, which were concentrated in the shallower water around the periphery and around organic floatage.

Activity in polluted waters was not affected

Table 2. Percent inhibition of successful adult emergence from pupal samples collected from 3 dairy wastewater lagoons before and following treatment with S-31183 at 0.1 kg (AI)/ha.

St. Clair Dairy (solids pond)		St. Clair Dairy (storage pond)		Jacobi Dairy	
Days after treatment	Percent inhibition	Days after treatment	Percent inhibition	Days after treatment	Percent inhibition
−1	0	−3	2	−1	2
0	1	−2	4	0	5
6	99	0	4	6	100
13	99	4	100	12	100
20	97	11	100	17	100
27	—[a]	19	79	24	96

[a] Water in lagoon was pumped down and could not be sampled.

by dilution, but continued even after up to 80% of the lagoon water was removed by pumping and replenished with untreated wastewater. This dilution occurred twice following the first and third applications to Nunes and resulted in no decrease in activity. Only after the third pumping, following the second Nunes application, was there any loss of activity, 35 days after treatment.

Pumping of wastewater from the lagoon would be expected to significantly reduce the concentration of pyriproxyfen-adsorbed, organic matter and thus biological activity. The fact we did not observe a reduction in biological activity indicated that most of the material remained in the lagoon, possibly accumulating in a concentrated band around the periphery or near the surface, as the water was drawn off from the bottom during pumping.

As was reported in our previous study, we were unable to recover pyriproxyfen from treated field waters after 48 h by chemical analysis, despite the continued presence of the material as demonstrated by biological activity. Further, this biological activity was greater in specimens allowed to complete development to pupae *in situ,* rather than collected as larvae and held in the laboratory, even when held in water from the collection site. Because of this and since the late fourth instar larva was the stage most sensitive to exposure to pyriproxyfen (Schaefer et al. 1988), mosquitoes should be allowed to pupate in the treated source before collection, after which they can be held in tap water. Collection of larvae instead of pupae may have caused the low activity of pyriproxyfen in dairy wastewater lagoons reported by Mulla and Darwazeh (1988), although the use of a lower rate [0.05 kg (AI)/ha] and different formulation (granular) may also have been factors.

Based on our study, it is inconclusive whether alternate applications of oil between applications of pyriproxyfen will prevent or diminish development of resistance. Larvicide oil was applied when it appeared that the pyriproxyfen effectiveness had diminished, based upon adult emergence in the pupal sample, as well as from the lagoon (observations), although residual control from the pyriproxyfen treatment often continued at a high level for extended periods after the initiation of the oil treatments. However, this time period when pyriproxyfen is not present in sufficient concentration to produce complete mortality will result in the elimination of only the most susceptible members of the population. Thus, treatments with an alternate chemical during this period are an important strategy. The challenge, then, is to utilize this valuable new agent without promoting resistance, understanding that each habitat may produce slightly different results in terms of length of residual activity.

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