FIELD EFFICACY OF SOME PROMISING MOSQUITO LARVICIDES AND THEIR EFFECTS ON NONTARGET ORGANISMS[1]

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ABSTRACT. A synthetic pyrethroid FMC-33297 [3-phenoxybenzyl(±)*cistrans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] was found to show exceptional activity against larvae and pupae of the mosquito *Culex peus* Speiser, *C. tarsalis* Coquillett, and *Aedes nigromaculis* Ludlow, under field conditions. It gave excellent control of the *Culex* species at 0.025–0.05 lb/acre, and good control of *Aedes* at 0.01–0.025 lb/acre. The organophosphates N-2596 (*O*-ethyl-*S*-*p*-chlorophenyl ethyl phosphonodithioate) S-2957 (diethyl-2, 5-dichloro-4-methylthiophenyl phosphorothionate) were also highly effective. S-2957 yielded excellent control of *Culex* at 0.025 lb/acre, while N-2596 yielded excellent control of OP-resistant *Aedes* at 0.05–0.10 lb/acre. The level of control was slightly better when less tolerant populations were treated.

FMC-33297 was not highly active against chironomid midge larvae, although suppressing tanypodine larvae for 2 weeks. S-2957 was highly effective against midge larvae. Mayfly naiads were severely affected by S-2957 and the higher rate of FMC-33297. Diving beetle larvae and adults were also slightly affected by both materials. Copepods were affected by S-2957 and the higher rate of FMC-33297, ostracods were only affected by the former and not the latter. Recovery of ostracods where affected was rapid.

INTRODUCTION

In recent studies (Darwazeh and Mulla 1974), a number of organophosphorus and synthetic pyrethroid insecticides were reported to show a high level of biological activity against various species of mosquito larvae and pupae in the laboratory and some of these also showed good activity under field conditions. In the field, the organophosphorus compounds performed well, but the efficacy of the pyrethroids was not commensurate with the activity as found in the laboratory. This difference in activity was attributed to their rapid rate of degradation under field conditions. Preliminary field evaluation of synthetic pyrethroids yielded some encouraging results in controlling adult *Aedes nigromaculis* (Ludlow) in irrigated pastures (Darwazeh and Mulla 1975).

The following studies were initiated to evaluate a new promising synthetic pyrethroid and other promising insecticides with high larvicidal activity (Darwazeh and Mulla 1974) against resistant and susceptible strains of mosquito larvae in the laboratory and under field conditions, and to determine their persistence in water as well as their effects on some nontarget organisms.

METHODS AND MATERIALS. The most active materials against larvae and pupae of *Culex pipiens quinquefasciatus* Say as found in the laboratory were selected for further field evaluation against susceptible and resistant strains of mosquito larvae. These materials were N-2596 (*O*-ethyl-*S*-*p*-chlorophenyl ethyl phosphonodithioate), FMC-33297 [3-phenoxybenzyl (±) *cis-trans*-3-(2,2-dichlorovinyl)-2,-dimethylcyclopropanecarboxylate], and S-2957 or OMS 1342 (diethyl 2,5-dichloro-4-methlthiophenyl phosphorothionate). Emulsifiable concentrate formulations were utilized in field tests. Tests were conducted in experimental ponds (12′ x 24′ and 12–15″ deep), located at the Aquatic Research Facility (Midgeville) of the University of California at Riverside, and in irrigated pastures in Kern and Tulare counties of California.

Ponds at Riverside were described by Mulla et al. (1970). The required amount of toxicant was mixed with 100 ml of tap

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water and applied from the side of the pond with an all-purpose (1-qt) household sprayer (Smart and Final Iris Co., Los Angeles, Calif. 90058). Each concentration was replicated twice, and in each test 2 ponds were left untreated as checks. Mosquito larval populations during May and June consisted of *Culex peus* Speiser while *Culex tarsalis* Coquillett was predominant during the tests in July and August. For larval and pupal assessment 5 dips per pond were taken prior to and 48 h after treatment, and % reduction was based on the number of 3rd- and 4th-stage larvae and pupae recovered by dipping prior to and after treatment.

Procedures for determining effects of these materials on mosquito larvae and nontarget organisms are described elsewhere (Fanara and Mulla 1974, Mulla et al. 1975). In brief, 5 straw bundles were placed in each pond, one in each corner and one in the middle. On each sampling date, one dip from the side of each straw bundle was taken, and all 5 dips were concentrated into one sample after removing excess water through 150-mesh stainless steel strainer cloth. The 5 dips composite sample was preserved with 95% ethyl alcohol, and all macro-organisms present in the sample were counted, examined microscopically and identified. The chironomid midge larval population was monitored by taking mud samples from the bottom of each pond. Two scoops per pond were taken with a 10-ft long telescopic handle attached at an adjustable angle to a 6″ x 6″ metal scoop. The mud samples were washed through a 50-mesh sieve and midge larvae were floated by using a saturated solution of $MgSO_4$ (Mulla et al. 1973b, 1974).

Tests in irrigated pastures were conducted in 1/32-acre plots. The required amount of toxicant was mixed with 1000 ml of water and applied with a ½-gal hand sprayer. In all tests, 2 replicates per application rate were used and 2 plots were left untreated as checks. Ten dips per plot were taken prior to, and 24 h after treatment. Mosquito larval population consisted of *Aedes nigromaculis* Ludlow. Larval susceptibility level to organophosphorus mosquito larvicides in test areas was determined earlier. Locations and field conditions of test plots are described in some of the tables. Percent reduction is based on the number of larvae and pupae recovered by dipping in the pre- and posttreatment counts.

RESULTS AND DISCUSSION

MOSQUITOES. The new pyrethroid FMC-33297 yielded excellent control of larvae and pupae of *Culex peus* Speiser (Table 1) in the experimental ponds. Its activity in general was similar against the 3rd- and 4th-stage larvae and pupae of this species. The material was very promising in the range of 0.025–0.05 lb/acre against immature stages of this mosquito. This is the first known pyrethroid (synthetic as well as natural) which has shown such high biological activity against mosquito larvae and pupae in field experiments. Other pyrethroids including pyrethrin, allethrin, dimethrin, and others did not show this level of activity against mosquitoes (Darwazeh and Mulla 1974, Mulla et al. 1973a).

In the same tests, the organophosphate material S-2957 showed good biological activity against larvae and pupae of this species. At the rate of 0.025 lb/acre this material yielded complete control of larvae and pupae of *Culex tarsalis* Coquillett for 9 days, but heavy populations of these appeared 13 days after treatment. This compound is characterized by high mammalian toxicity (5 mg/Kg acute oral LD_{50}/rat) and showed some toxicity to some nontarget organisms in this study (see Tables 5–7).

The pyrethroid FMC-33297 also showed exceptional activity against larvae and pupae of *C. tarsalis* in the experimental field ponds at Midgeville (Table 2). At the higher rate (0.1 lb/acre) almost complete control of larvae and pupae was obtained for a period of 9 days. However, complete recovery of larval and pupal

Table 1. Effectiveness of the synthetic pyrethroid FMC-33297 (EC 0.8) against larvae and pupae of *Culex peus* in experimental ponds (Midgeville, June 1974).

Rate lb/acre	Avg. no. larvae and pupae/5 dips				(%) Reduction	
	Pretreat		Post-treat (48 hr)			
	L	P	L	P	L	P
			Test 1			
0.010	40	14	24	12	40	14
0.025	21	9	3	3	86	67
0.050	31	21	2	1	94	95
Check	9	1	24	1	0	0
			Test 2			
0.050	17	2	1	0	94	100
0.100	29	23	0	0	100	100
Check	7	4	11	2	0	0
			Test 3			
0.100	45	4	0	0	100	100
0.250	34	2	0	0	100	100
Check	60	2	64	8	0	0

populations was realized 13 days after treatment. The recovery was much higher at the lower dosage than at the higher.

Several tests were conducted in the field against larvae of the pasture mosquito *Ae. nigromaculis* using the pyrethroid FMC-33297 and the organophosphate N-2596. The susceptibility levels of larvae of this species to commercial larvicides are presented in Table 3. It is apparent that the populations in Tulare county are highly resistant to parathion and methyl parathion, also showing some degree of tolerance to fenthion. The populations in the Kern county test area were also tolerant to parathion and methyl parathion, but quite susceptible to fenthion and chlorpyrifos.

Four experiments were conducted against these OP-resistant populations of *Ae. nigromaculis*. In the first test FMC-33297 yielded complete control of larvae (and pupae which appeared 24 h post treatment) of a highly OP-resistant strain at the rate of 0.05 lb/acre and higher (Table 4). The organophosphate N-2596 gave excellent control of larvae but not pupae at the highest rate (0.1 lb/acre).

In the second test against the same population, FMC-33297 was used at lower rates than in the previous experiment (see Table 4). At the rate of 0.01 lb/acre this

Table 2. Effectiveness of the pyrethroid FMC-33297 (EC 0.8) against larvae and pupae of *Culex tarsalis* in experimental ponds (Midgeville, September 1974).

Rate lb/acre	Avg. no. larvae and pupae/5 dips											
				Post-treatment (days)								
	Pretreatment			2			6			9[a]		
	1–2	3–4	P	1–2	3–4	P	1–2	3–4	P	1–2	3–4	P
0.05	35	19	4	8	6	1	9	3	2	39	20	1
0.10	67	15	1	1	2	1	2	0	0	18	3	0
Check	34	38	2	48	34	4	88	32	10	77	33	2

[a] Larvae and pupae present in large numbers 13 days post-treatment.

Table 3. Susceptibility level of 4th-stage *Ae. nigromaculis* larvae to various OP larvicides in plots used for the evaluation of new materials.[a]

Larvicide	Fornasero Pasture Tulare County LC50	Fornasero Pasture Tulare County LC90	Kings Truck Co. Tulare County LC50	Kings Truck Co. Tulare County LC90	Rancho Santa Maria Kern County LC50	Rancho Santa Maria Kern County LC90
Parathion	0.37	0.98	>0.45	b	0.095	0.150
Me. parathion	0.29	0.63	>0.30	b	0.042	0.180
Fenthion	0.035	0.09	0.03	b	0.004	0.009
Chlorpyrifos	....	....	0.007	b	0.002	0.005

[a] Data obtained from Calif. State Health Department, Fresno, Calif.
[b] Data not available.

material produced complete control of larvae. N-2596 at the 2 rates (0.05 and 0.1 lb/acre) produced excellent control of larvae. Since the larvae at the time of the treatment were early 4th's, no pupation occurred up to the post-treatment time.

The next 2 tests were conducted in Kern county (Rancho Santa Maria) where the *Aedes* larvae were somewhat less tolerant to some OP larvicides. Initially both FMC-33297 and N-2596 were applied at lower rates. Both materials yield excellent if not complete control of the larvae and pupae. N-2596 performed better against these less resistant larvae than those in the Fornasero pasture (see Table 4). In the succeeding test, the rates of both compounds were increased. Results with FMC-33297 were erratic in this test, but N-2596 yielded good if not complete control of the larvae and pupae. Heavy vegetation and lack of penetration of spray from manually applied treatment could have contributed to this pattern of results. The erratic and poor results as seen in test 3 with FMC-33297 were likely due to heavier vegetation canopy in the treated plots and, therefore, lack of adequate penetration by the sprays. All three materials evaluated were quick-acting, bulk of the mortality occurring within 4–6 h after treatment.

NONTARGET ORGANISMS. Chironomid midges, although at times creating a nuisance problem, are considered here as nontarget organisms. Both the tanypodine and chironomine midge larvae were slightly depressed by the lower rate (0.05 lb/acre) of FMC-33297 (Table 5). At the higher rate (0.1 lb/acre) the effect was much greater for the duration of the experiment on both groups, but more severe on the tanypodine group. This latter group in general is less susceptible to OP insecticides (Mulla et al. 1973b). S-2957 at both rates suppressed both groups of midges. The level of suppression was higher for the tanypodines. It appears that this compound could be employed in midge control where this is desired. However, its high mammalian toxicity and toxicity to nontarget organisms are some of the drawbacks one has to deal with.

Mayfly naiads and diving beetle larvae and adults were affected by both compounds at the rates employed here (Table 6). There was no noticeable recovery at any of the dosages except the lower and practical dosage of FMC-33297 (where mayfly naiads recovered) up to 16 days after treatment. The numbers of diving beetle larvae and adults, however, were quite low and, therefore, no hard and fast conclusions can be drawn.

Copepods and ostracods were not noticeably affected by the lower and practical rate (0.05 lb/acre) of FMC-33297 for a sustained period (Table 7). At the higher rate the copepods sustained a lower population during the duration of the experiment. Ostracods in general were not affected by either rate of the compound for any length of time.

Table 4. Effectiveness of two experimental larvicides against OP-resistant larvae of *Ae. nigromaculis* in irrigated pastures (1974).

Material and formulation	Rate lb/acre	Avg. no. larvae and pupae/dip				% control
		Pretreatment		Post-treatment		
		L	P	L	P	
		1—Fornasero pasture,[a] Tulare Co. (Aug. 28)				
FMC-33297 (EC 0.8)	0.05	71	0	0	0	100
	0.10	43	0	0	0	100
	0.25	35	0	0	0	100
N-2596 (EC 4)	0.01	22	0	20	6	0
	0.05	34	0	4	3	79
	0.1	59	0	2	3	92
Check	....	31	0	31	5	0
		2—Fornasero pasture,[b] Tulare Co. (Aug. 29)				
FMC-33297 (EC 0.8)	0.005	11	0	1	0	91
	0.01	40	0	0	0	100
	0.025[c]	65	0	0	0	100
N-2596 (EC 4)	0.05	55	0	1	0	98
	0.10	18	0	1	0	95
Check	....	17	0	24	0	0
		3—Rancho Santa Maria pastures,[d] Kern Co. (Sept. 17)				
FMC-33297 (EC 0.8)	0.005	49	0	8	1	82
	0.01	37	0	2	3	86
N-2596 (EC 4)	0.01	81	0	0	3	96
	0.025	80	0	1	1	97
Check	....	47	0	38	18	0
		4—Rancho Santa Maria pastures,[d] Kern Co. (Sept. 19)				
FMC-33297 (EC 0.8)	0.01	22	1	1	2	87
	0.025	33	1	4	6	71
	0.05	16	2	4	3	62
N-2596 (EC 4)	0.05	17	1	0	2	81
	0.10	23	1	0	1	96
Check	....	27	0	38	15	0

[a] Vegetation short, no penetration problem, water depth 6–8 inches. Larvae were late 4th-stage, pupation occurred soon after treatment.
[b] Larvae early 4th-stage.
[c] Diving beetle adults dead.
[d] Vegetation dense and lodged.

S-2957 depressed populations of copepods at both rates (see Table 7). It also slightly depressed the ostracod populations for a period of 9 days or so; thereafter, the population recovered to the pretreatment levels.

From these studies, it appears that both FMC-33297 and S-2957 affect mayfly naiads severely, but these recovered within 2 weeks or so at the practical rate of application (0.05 lb/acre) of the former. Against the remaining groups, the former compound was less hazardous than the latter compound. Against chironomid midges, the latter compound is more effective. Dragonfly naiad mortality was considerable, especially at the higher rate of application of both materials.

Table 5. Effectiveness of mosquito larvicides on chironomid midge larvae in experimental ponds (Midgeville, Sept. 1974).

		Avg. no. chironomid midge larvae/sample pre- and post-treat (days)											
		Tanypodinae[a]						Chironominae[b]					
Material and formualtion	lb/acre	Pre	2	6	9	13	16	Pre	2	6	9	13	16
FMC-33297 (EC 0.8)	0.05	24	8	3	13	10	9	10	6	4	4	14	7
	0.10	28	3	1	9	5	7	7	2	5	4	2	5
S-2957 ((EC 4)	0.05	18	0	0	1	7	0	7	0	3	4	0	10
	0.10	5	0	0	0	1	0	8	0	0	3	1	7
Check	....	13	21	10	18	3	18	4	3	5	4	14	4

[a] Mostly *Pentaneura* and *Tanypus*.
[b] Mostly *Tanytarsus* and *Chironomus*.

Table 6. Effect of mosquito larvicides on nontarget insects in experimental ponds (Midgeville, Sept. 1974).

		Avg. no. nontarget insects/5dips composite sample pre- and post-treat (days)											
		Ephemeroptera[b]						Coleoptera[c]					
Material and formualtion	lb/acre	Pre	2	6	9	13	16	Pre	2	6	9	13	16
FMC-33297 EC (0.8)	0.05	8	0	0	0	1	9	1	2	0	0	0	0
	0.10	12	0	0	0	1	1	1	3	0	1	0	0
S-2957 (EC 4)	0.05	12	0	1	0	2	2	2	1	0	0	1	0
	0.10[a]	20	0	1	1	0	1	4	3	2	0	0	2
Check	....	16	17	13	12	12	14	2	2	1	1	2	1

[a] Dragonfly naiads were observed dead in the ponds in large numbers 2 days after treatment.
[b] Ephemeroptera, Baetidae, mostly *Baetis* sp.
[c] Coleoptera, Hydrophilidae, and Dytiscidae, larvae and adults.

Table 7. Effect of mosquito larvicides on crustacean populations in experimental ponds (Midgeville, Sept. 1974).

		Avg. no. organisms/5 dips composite sample pre- and post-treat (days)											
		Copepoda[a]						Ostracoda[b]					
Material and formualtion	lb/acre	Pre	2	6	9	13	16	Pre	2	6	9	13	16
FMC-33297 (EC 0.8)	0.05	24	2	14	11	60	25	7	2	4	11	17	25
	0.10	10	0	2	1	7	4	18	1	6	15	12	28
S-2957 (EC 4)	0.05	4	0	1	1	1	0	6	2	1	1	5	6
	0.10	7	0	1	2	5	6	5	3	1	3	2	5
Check	....	10	12	13	7	6	12	5	5	7	4	12	7

[a] Mostly *Cyclops* and *Diaptomus*.
[b] Mostly *Cypricercus* and *Cyrinotus*.

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