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Impact of Selected Synthetic Pyrethroids and Organophosphorous Pesticides on the Tadpole Shrimp, *Triops longicaudatus* (Le Conte) (Notostraca: Triopsidae)

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Predation by the omnivorous tadpole shrimp, Triops longicaudatus (LeConte) (Notostraca: Triopsidae), reduces larval populations of the mosquito Culex tarsalis Coquillett in experimental ponds in the Coachella Valley of southern California and has made field evaluation of other larval control agents very difficult. T. longicaudatus is also a pest in rice fields where its digging and feeding activities uproot young seedlings, may retard seedling growth, and damage seeds (Anonymous 1983, Grigarick et al. 1985). In rice fields, this crustacean is controlled primarily by applying copper sulfate (Grigarick et al. 1985). Holck and Meek (1987) found that another crustacean rice field pest, the red swamp crawfish, Procambarus clarkii Girard (Decapoda: Cambaridae), was most susceptible to resmethrin + piperonyl butoxide (PBO) (LC_{50}: 0.00082 mg/L) as compared to several other pesticides used commonly for mosquito control. Our objectives were to identify a material that would control tadpole shrimp but would not exhibit residual activity that would limit mosquito populations and interfere with field tests of larval control agents. Here, we report the results of field tests and laboratory bioassays with two synthetic pyrethroids and two organophosphorous pesticides against tadpole shrimp.

MATERIALS AND METHODS

Field tests were conducted in 30-36 m^2 ponds at the University of California-Riverside, Aquatic and Vector Control Research Facility in Oasis, California. The ponds were filled with well water and depth was maintained at 30 cm by float valves. Water temperature was monitored with a minimum-maximum thermometer. Mulla et al. (1982) have provided a detailed description of the site.

The required amount of resmethrin (oil concentrate: resmethrin 18%, piperonyl butoxide 56%, inert ingredients 26%; Lot no. 2150GJM-14) was diluted in 50 mL isopropyl alcohol and applied to the experimental ponds with plastic squeeze bottles at a rate of 0.028, 0.056 or 0.112 kg active ingredient (AI)/ha on 4 and 8 d after flooding. At 4 d after flooding, three replicates were used per application rate (0.028 and 0.056 kg AI/ha) and as controls

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(untreated ponds). Similar replication was used during tests conducted at 8 d after flooding; however, tadpole shrimp were found at sufficient densities in only two control ponds.

Prior to application, cypermethrin (Emulsifiable Concentrate (EC) 2.5; Lot no. E2415-22) was diluted to a 1% solution in acetone. The required amounts of the synthetic pyrethroid pesticide, cypermethrin, and the organophosphorous pesticides, chlorpyrifos (EC 4) and fenthion (EC 4), were diluted in 50 mL distilled water and applied to the ponds with plastic squeeze bottles. Cypermethrin was applied at rates of 0.0001, 0.0003, 0.0006, 0.001, 0.003, 0.012 and 0.056 kg AI/ha at 4 d after flooding. Chlorpyrifos and fenthion were applied at 4 or 6 d after flooding at rates of 0.006, 0.011, 0.028, 0.056 and 0.112 kg AI/ha, and 0.056 and 0.112 kg AI/ha, respectively. During the 1988 studies, controls were run in triplicate; however, pesticide treatments were only duplicated. During 1989, all pesticide treatments were triplicated. All posttreatment samples were taken 3 d after pesticide application.

Emulsifiable concentrates are not diluted customarily with acetone. In order to determine whether the acetone dilution enhanced cypermethrin activity, we compared the EC diluted with acetone vs dilutions with only distilled water. Cypermethrin was applied as above at rates of 0.0001, 0.0003, 0.0006 and 0.0011 kg AI/ha at 4 d after flooding. Each treatment was triplicated and posttreatment samples were taken two days after application.

Tadpole shrimp were sampled with either a dip net (diameter: 30 cm; mesh: 8 openings/cm) or a D-frame aquatic net (base width: 33 cm; mesh: 11 openings/cm) along the perimeter of each pond. The type of net used was consistent within a given test. Two hauls per pond, each equal to one-half the pond perimeter, were placed individually into enamel pans. Tadpole shrimp were counted and replaced into their respective ponds. For reasons elaborated upon below, shrimp were arbitrarily divided into two size categories: large (> 1.0 cm length excluding the cercopods) and small (< 1.0 cm length excluding the cercopods). However, large shrimp were typically greater than 1.7 cm in length (excluding the cercopods) and the length of small shrimp was only several millimeters.

To determine the impact of pesticides against Triops, percent reduction was calculated using the Mulla formula (Mulla et al. 1971): % reduction = [(100)(C1 x T2)/(T1 x C2)], where C1 is the number of shrimp in the control pretreatment; C2 is the number of shrimp in the control posttreatment; T1 is the number of shrimp in the treated pretreatment; and T2 is the number of shrimp in the treated posttreatment. This formula has been used in situations where abundance fluctuated in the control ponds (Mulla et al. 1971). Pretreatment counts were examined statistically by analysis of variance (ANOVA) and Duncan's Multiple Range test. Statistical examination of posttreatment counts by ANOVA was often inappropriate because the mean and variance were zero for some treatments. Therefore, the Mann-Whitney U statistic was used to compare the tadpole shrimp counts in each pairwise treatment combination. The limit of statistical detection was p=0.10 where treatments were duplicated and triplicated. For tests in which all treatments were triplicated, the limit of statistical detection was p=0.05.

The toxicities of the four pesticides also were determined in the laboratory. Field-collected tadpole shrimp (4-5 d old) were placed individually into 4-oz disposable cups (Sweetheart Cup Division, Baltimore, Maryland) containing 100 mL of well water. Stock solutions of the pesticides were made in acetone, serially diluted, and the required amount of toxicant solution was added to each of ten replicate cups. One mL of acetone was added to each control. After 24 hr at 28±2°C, mortalities were recorded and subjected to probit analysis. The Statistical Analysis System (SAS) was used to obtain the LC_{50} and LC_{90} (± 95% fiducial limits) for each pesticide. Carinal lengths were measured mid-dorsally for 30 tadpole shrimp in each test.

RESULTS AND DISCUSSION

Resmethrin applied 4 d after pond flooding at rates of 0.028 and 0.056 kg AI/ha reduced tadpole shrimp abundance by 35 and 87%, respectively, as compared to untreated ponds (Table 1). Because small-sized tadpole shrimp were found in many of the treated ponds, treatments did not differ significantly. On day 7, small-sized individuals were observed in all ponds without large shrimp: two replicates at 0.028 kg AI/ha and three replicates at 0.056 kg AI/ha.

Hurlbert (1969) and Kerfoot and Lynch (1987) suggested that chlorpyrifos and rotenone stimulated hatching of tadpole shrimp eggs. However, in the latter case, fluctuating water levels might have caused these results. Although most Triops eggs hatch 1 to 3 d after habitat flooding, hatching may continue for 1 to 2 wk (Anonymous 1983, Grigarick et al. 1985). We attribute the lack of control in our test, and the preponderance of small-sized shrimp, to the effects of slow inundation and post application hatching rather than stimulation of egg hatching by resmethrin. Continued hatching from dormant eggs in the substrate that were not affected by the short-lived, pyrethroid pesticide (Elliot 1977) resulted in populations of small-sized tadpole shrimp. In ponds containing only large-sized shrimp, cannibalism probably eliminated the late recruits. When comparisons were based only on large-sized tadpole shrimp, Triops abundance was reduced significantly by resmethrin applied at 0.056 kg AI/ha (Table 1).

Resmethrin applied 8 d after flooding reduced T. longicaudatus numbers by > 90% (Table 1). On day 11, tadpole shrimp were present in two of the three replicates at the two lowest rates. Resmethrin provided complete control only at the rate of 0.112 kg AI/ha, and the large percent reductions of Triops at the two lowest rates are an artifact of the large increase of Triops in the untreated ponds (Table 1). As compared to the untreated ponds,

Table 1. Control of *Triops longicaudatus* with various pesticides in experimental ponds.

Material (Formulation)	Application: Days after Flooding	Application: Rate (kg/ha)	Total No. of Tadpole Shrimp in 2 Hauls: Pre-treatment†	Total No. of Tadpole Shrimp in 2 Hauls: Post-treatment†	Percent Reduction
Resmethrin (oil concentrate: 18%)	4*	0.028	96 a	76 a	35
		0.056	176 a	29 a	87
		Control	54 a	66 a	-
	4*††	0.028	96 a	26 ab	78
		0.056	176 a	0 b	100
		Control	54 a	66 a	-
	8*	0.028	88 b	81 b	93
		0.056	334 a	17 b	99
		0.112	109 b	0 b	100
		Control	13 c	160 a	-
Cypermethrin (EC 2.5)	4*****	0.0001	198 a	177 a	5
		0.0003	204 a	27 b	86
		0.0006	165 a	0 c	100
		Control	141 a	132 a	-
	4****	0.0006	21 a	0 a	100
		0.001	33 a	0 a	100
		Control	51 a	98 b	-
	4***	0.001	24 a	0 a	100
		0.003	46 a	0 a	100
		Control	8 a	14 b	-
	4**	0.012	92 a	0 a	100
		0.056	253 a	0 a	100
		Control	114 a	57 b	-
Fenthion (EC 4)	4**	0.056	50 a	13 a	48
		0.112	145 a	4 a	94
		Control	114 a	57 b	-
Chlorpyrifos (EC 4)	6******	0.006	69 a	147 a	32
		0.011	78 a	24 b	90
		Control	51 a	159 a	-
	4******	0.011	132 a	36 b	81
		0.028	192 a	0 c	100
		Control	165 a	231 a	-
	4****	0.028	27 a	0 a	100
		0.056	10 a	0 a	100
		Control	51 a	98 b	-

Table 1. Continued.

*	Tested 23 May-4 June 1988; water temp. range: 23-32°C.
**	Tested 30 June-4 July 1988; water temp. range: 25-36°C.
***	Tested 11 Aug.-15 Aug. 1988; water temp. range: 22-33°C.
****	Tested 30 Sept.-6 Oct. 1988; water temp. range: 21-30°C.
*****	Tested 27 Apr.-4 May 1989; water temp. range: 18-28°C.
******	Tested 2 May-11 May 1989; water temp. range: 21-32°C.
†	Treatments followed by the same letter do not differ significantly by Duncan's Multiple Range test ($p > 0.05$) or by the Mann-Whitney U test ($p = 0.05$ or $p = 0.10$; see text). Comparsions were made for each pesticide within each test.
††	Comparisons based only on large-sized tadpole shrimp.

Triops abundance was reduced significantly in all resmethrin treatments (Table 1: Mann-Whitney $U_{3,2}$, $p = 0.10$). Unlike the first test, small-sized individuals were virtually absent from treated ponds. Only two small-sized individuals (approximately 2 days old) were observed in one replicate treated at 0.028 kg AI/ha.

Cypermethrin was the most effective compound of the pesticides that we tested against _T. longicaudatus_ and provided complete control at extremely low application dosages (0.0006 kg AI/ha: Table 1). Pairwise comparisons between treatments showed that shrimp abundance differed significantly in treated ($\geq$ 0.0003 kg AI/ha) and untreated ponds (Mann-Whitney $U_{3,3}$, $p = 0.05$ or $U_{3,2}$, $p = 0.10$). Small-sized tadpole shrimp were never observed in tests using cypermethrin, chlorpyrifos and fenthion.

As compared to dilutions with only distilled water, cypermethrin activity against tadpole shrimp was enhanced by diluting the EC formulation with acetone (Table 2). Tadpole shrimp were reduced >90% by acetone-diluted cypermethrin applied at $\geq$ 0.0003 kg AI/ha. At similar rates, cypermethrin diluted only with water reduced shrimp numbers between 48 and 98%, and levels of control varied between the tests (e.g., 0.0006 kg AI/ha).

Tadpole shrimp abundance on day 7 after flooding differed significantly between untreated ponds and ponds treated with chlorpyrifos or fenthion (Mann-Whitney $U_{3,3}$, $p = 0.05$ or $U_{3,2}$, $p = 0.10$). Chlorpyrifos applied at $\geq$ 0.028 kg AI/ha provided complete control of tadpole shrimp (Table 1). Fenthion applied at 0.056 and 0.112 kg AI/ha reduced tadpole shrimp numbers by 48 and 94%, respectively (Table 1).

As compared to organophosphorous pesticides, synthetic pyrethroids such as cypermethrin are preferred control agents because they exhibit higher insecticidal activities, are less toxic to mammals and have shorter lifetimes. Cypermethrin was 10 times more active

Table 2. Effect of cypermethrin on Triops longicaudatus in experimental ponds.

Rate (kg/ha)	Total No. of Tadpole Shrimp in 2 Hauls: Pretreatment	Total No. of Tadpole Shrimp in 2 Hauls: Posttreatment	Percent Reduction
		EC in acetone	
0.0001	162 a†	90 a†	38
0.0003	135 a	13 b	93
0.0006	183 a	0 b	100
Control*	216 a	192 a	-
		EC in water	
0.0001	156 a	78 a	44
0.0003	135 a	48 a	60
0.0006	123 a	56 a	48
Control*	216 a	192 a	-
0.0006	27 a	2 a	98
0.0011	22 a	0 a	100
Control**	11 a	44 b	-

*Tested 12-17 June 1989; water temp. range: 24-35°C.
**Tested 21-26 July 1989; water temp. range: 26-35°C.
†Counts in a column followed by the same letter do not differ significantly ($p > 0.05$) within a test by Duncan's Multiple Range Test (pretreatment) or Mann-Whitney U test (posttreatment).

Table 3. Toxicities of pyrethroid and organophosphorus pesticides to Triops longicaudatus at 28±2°C.

Pesticide	LC_{50} (μg/L)	LC_{90} (μg/L)	Carinal Length (cm: $\bar{x} \pm 1$ SD)
Chlorpyrifos	4.0 (1.7-4.9)[a]	7.5 (6.4-11.1)[a]	0.80±0.07
Fenthion	73.8 (63.5-87.1)	114.8 (98.1-151.4)	0.80±0.07
Cypermethrin	0.084 (0.072-0.107)	0.134 (0.111-0.197)	0.77±0.06
Resmethrin	0.7 (0.5-0.9)	1.1 (0.9-1.6)	0.79±0.07

[a]95% Fiducial limits.

than resmethrin, and 50 to 1000 times more active than the organophosphorus pesticides, against 4- to 5-d-old T. longicaudatus in the laboratory (Table 3). Relative activities of the pesticides were similar in the field tests (Table 1). Large mosquito populations were observed one week after pesticide applications and subsequent macroinvertebrate colonization of treated ponds was similar to that observed in untreated ponds which lacked T. longicaudatus. The absence of mosquito larvae in most of the untreated ponds that contained tadpole shrimp (personal observation) documents further Triops' potential as a biological control agent for mosquitoes.

Applying resmethrin or chlorpyrifos at a rate between 0.056 and 0.112 kg AI/ha approximately 1 wk after habitat flooding also will control T. longicaudatus. Fenthion provided significant control at only 0.112 kg AI/ha. Similar application rates were suggested during the mid-1960's for parathion in rice fields (0.112 kg AI/ha); however, resistance to parathion in T. longicaudatus was observed during the 1970's and 80's (Grigarick et al. 1985). We do not know whether the cross resistance to pyrethroids observed for insects occurs in tadpole shrimp. While the pesticides tested in our study might be used to control Triops, given the likelihood that tadpole shrimp will also evolve resistance to them, recommendations by Grigarick et al. (1985) probably pertain here as well.

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