DOSE-MORTALITY RESPONSES OF CRAWFISH AND MOSQUITOES TO SELECTED PESTICIDES[1,2]

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ABSTRACT. A study was conducted to determine the toxicities (LC_{50}s) of several pesticides on the commercially important red swamp crawfish, *Procambarus clarkii,* and 3 mosquito species common in Louisiana ricelands—*Anopheles quadrimaculatus, Culex salinarius* and *Psorophora columbiae.* Pesticides tested in laboratory bioassays included *Bacillus sphaericus, B. thuringiensis* var. *israelensis,* bendiocarb, glyphosate, isostearyl alcohol, malathion, propoxur, resmethrin synergized with piperonyl butoxide (PBO) and thiobencarb. Isostearyl alcohol was the least toxic compound to crawfish, with a LC_{50} of >10,000 ppm, while resmethrin + PBO (1:3 ratio) was the most toxic with a LC_{50} of 0.00082 ppm. The herbicides glyphosate and thiobencarb were the least toxic compounds for the mosquito species tested, while *B. t.* var. *israelensis* and resmethrin + PBO were the most toxic.

INTRODUCTION

The red swamp crawfish, *Procambarus clarkii* Girard (Decapoda: Cambaridae), is an economically important, non-target arthropod that is cultivated in riceland areas where parish-wide mosquito control operations are often conducted. Pesticides commonly used by mosquito control agencies operating in these areas include malathion and resmethrin for adult control. While a diesel oil/triton mixture was often used in the past for larval control, many mosquito control operations are now considering switching to biological agents such as *Bacillus thuringiensis* var. *israelensis* and *B. sphaericus,* or monomolecular surface films such as isostearyl alcohol. Agricultural pesticide applicators and mosquito abatement personnel have expressed their concern about the potential risk that various pesticides pose to commercial crawfish populations. For this purpose, a study was conducted to assess the toxicities of selected pesticides to the red swamp crawfish.

MATERIALS AND METHODS

Laboratory experiments were conducted to evaluate the efficacies of selected bacterial and chemical pesticides that are currently labeled for use in the riceland agroecosystem of Louisiana. Technical grade pesticides generally were not used in order that a more relevant evaluation could be made for these pesticides as they are used in field conditions. Pesticides containing *Bacillus sphaericus* Neide and *B. thuringiensis* var. *israelensis* (serovar H-14) de Barjac were the only biological representatives in the series of tests. The active ingredients of chemical pesticides tested included bendiocarb, glyphosate, isostearyl alcohol, malathion, propoxur, resmethrin + piperonyl butoxide (PBO) and thiobencarb. The trade names and other pertinent information about these compounds are provided in Table 1.

These pesticides were evaluated against three species of mosquitoes that commonly use rice fields and associated pasturelands as larval habitats. The species included 2 permanent water mosquitoes, *Anopheles quadrimaculatus* Say and *Culex salinarius* Coquillett; and a riceland floodwater mosquito, *Psorophora columbiae* (Dyar and Knab). These data were compared with the toxicological effects of the same pesticides on the red swamp crawfish, *P. clarkii.*

The toxicological method used for *P. clarkii* closely followed the U. S. Environmental Protection Agency guidelines for evaluating the effects of potential toxicants on aquatic organisms (Stephen 1975). Modifications to the guidelines were made according to de la Bretonne et al. (1969) and Cheah et al. (1980). These changes involved the adjustment of test water hardness with calcium chloride and lining of aquaria with polyethylene bags. The water hardness was standardized to minimize stress on the crawfish, and the 0.05 mm thick, 20 × 60 cm polyethylene bags provided refuge for the crawfish to prevent cannabalism. Immature *P. clarkii* (25–40 mm long) were collected from crawfish-rearing ponds and associated roadside ditches on the

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Table 1. Common names, trade names, uses and formulations of pesticides tested in the laboratory against mosquito larvae and crawfish.

Common name[1]	Trade name	Use	Formulation
Bacillus sphaericus	BSP-1	Insecticide	Flowable concentrate (14%)
Bacillus thuringiensis var. *israelensis*	Bactimos	Insecticide	Flowable concentrate (14%)
bendiocarb	Ficam	Insecticide	Technical
glyphosate	Roundup	Herbicide	Soluble liquid (1.5 lb/gal)
isostearyl alcohol	Arosurf	Insecticide	Technical
malathion	Cythion	Insecticide	Oil concentrate (95%)
propoxur	Baygon	Insecticide	Emulsifiable concentrate (1.5 lb/gal)
resmethrin + piperonyl butoxide (1:3 ratio)	Scourge II	Insecticide	Oil concentrate (16% + 54%)
thiobencarb	Bolero	Herbicide	Emulsifiable concentrate (8 lb/gal)

[1] Common names of agrichemicals listed are taken from in C. R. Worthing (ed.) 1983. The pesticide manual, a world compendium (7th ed.). Lavenham Press Ltd. Lavenham, U.K.

Louisiana Agricultural Experiment Station, LSU Agricultural Center, Ben Hur Farm near Baton Rouge, Louisiana. Crawfish were held for 24–72 hr prior to treatment in two 75-liter aquaria. The aquaria were partially filled with tapwater, adjusted to a total hardness of 100 mg/liter with $CaCl_3$ and continuously aerated.

Twenty-four aquaria (20-liter capacity each) were used for each test. This allowed for 4 replications of 5 pesticide concentrations and a control within each replication. Ten immature crawfish were placed in each lined aquarium, which had been filled with 5 liters of tapwater and aerated for 12 hr prior to the test. The aquaria were not aerated during the test. Dosage selection was determined in the iterative manner as discussed by Robertson et al. (1984). Mortality of the crawfish was recorded at 96 hr posttreatment. Data were subjected to a probit analysis computer program, (Micro Probit©) developed by Sparks and Sparks (1982)[4], to calculate the LC_{50} values for each pesticide.

Pesticide toxicity tests were conducted on fourth instar mosquito larvae using a combination of techniques as described by Mulla et al. (1982), World Health Organization (1963), and Van Essen and Hembree (1980). Glass beakers (600-ml capacity) filled with 500 ml of distilled water were used as test containers. The deviation from the World Health Organization standardized toxicological procedures was necessitated when repeated excessive control mortality of the larvae was observed for *Ps. columbiae.* A minimum of 4 pesticide concentrations plus one control were replicated 4 times, with 10 larvae placed in each beaker prior to the introduction of pesticide. Larvae of *Cx. salinarius* and *An. quadrimaculatus* were obtained from the LSU laboratory colony that had recently been established from wild-caught populations of both species. *Psorophora columbiae* larvae were reared from eggs deposited by adult females collected from Vermilion Parish, Louisiana. The number of moribund and dead individuals was recorded 24 hr posttreatment. For isostearyl alcohol, additional tests were conducted with mortality recorded at 72 hr posttreatment. This compound exerts its maximum toxicity on immature mosquitoes at a period >24 hr following exposure. In these prolonged tests, larvae were initially fed a mixture of liver powder, ground rodent chow, brewer's yeast and distilled water. Equal parts of the dry ingredients were mixed together to make 5 g of powder, which was subsequently added to 100 ml of distilled water. One ml of the slurry was pipetted into each beaker. Mosquito mortality data were subjected to the same probit analysis program as discussed previously regarding the crawfish tests.

[4] Micro Probit©: probit analysis by the method of Finney (1971). Sparks, T. and S. Sparks. 1982. Department of Entomology, Louisiana State Univ., Baton Rouge, LA 70803.

RESULTS AND DISCUSSION

Probit analyses of toxicities for selected bacterial and chemical pesticides against the red swamp crawfish are summarized in Table 2. Laboratory tests indicated that isostearyl alcohol was the least toxic compound tested against crawfish, with a LC_{50} of >10,000 ppm. The bacterial agents *B. t. israelensis* and *B. sphaericus* were the next least toxic with a LC_{50} of 103 ppm and 75 ppm, respectively. These dosage rates were at least 500 times greater than the maximum labeled rates for field applications of these bacterial agents to control mosquitoes.

Malathion and glyphosate exhibited similar toxicities to immature crawfish in these laboratory tests. Previously published studies also report similar LC_{50}s for these compounds. Wellborn et al. (1984) listed a LC_{50} value of 50 ppm for malathion on *P. clarkii*. Folmar et al. (1979) determined the LC_{50} for the herbicide glyphosate on freshwater scuds (Crustacea: Amphipoda) to be 43 ppm.

The most toxic herbicide evaluated was the thiocarbamate, thiobencarb. The other carbamates that were evaluated included the insecticides propoxur and bendiocarb. These latter compounds ranked second and third in their toxicities to crawfish used in these laboratory tests.

Resmethrin, synergized with piperonyl butoxide at a ratio of 1:3, respectively, was the most toxic pesticide evaluated with a LC_{50} of 0.00082 ppm. The toxicity of this mixture corresponded closely with the LC_{50} of 0.0004 ppm for the pyrethroid permethrin against *Procambarus* spp. (Wellborn et al. 1984).

The results of these tests conducted against fourth instar *An. quadrimaculatus*, *Cx. salinarius* and *Ps. columbiae* are presented in Tables 3, 4 and 5, respectively. In general, the herbicides glyphosate and thiobencarb were the least toxic, whereas *B. t.* var. *israelensis* and resmethrin + PBO was the most toxic. LC_{50} values for the other chemicals assayed varied among species.

These test results against mosquito larvae are comparable to results obtained in other toxicological studies involving mosquito larvae. LC_{50}s between 0.013 ppm and 0.126 ppm were determined for various strains of *B. sphaericus* on *Ps. columbiae* (Mulla et al. 1985). The LC_{50}s for malathion (0.076 ppm) and propoxur (0.2 ppm) were determined on susceptible *Cx. quinquefasciatus* Say (El-Khatib and Georghiou 1985). These results are near the same values as those reported in this study for *Cx. salinarius*. The high LC_{50} values obtained for mosquito larvae exposed to the herbicide glyphosate were unexpected. A LC_{50} of 55.0 ppm for glyphosate on chironomid midges (Diptera: Chironomidae) was reported by Folmar et al. (1979). Nonetheless, these results were consistent over several trials. Other than the taxonomic difference, no explanation is offered for this apparent discrepancy.

The ratios of pesticide toxicities for mosquito larvae and crawfish are given in Table 6. This selectivity ratio was calculated for each pesticide

Table 2. Ninety-six hour probit analyses of toxicities for selected bacterial and chemical pesticides against the red swamp crawfish, *Procambarus clarkii* (Decapoda: Cambaridae).[1]

Pesticide	LC_{50} (ppm)	95% fiducial limits		Slope
		Lower	Upper	
Bacillus sphaericus	75.19	69.49	80.22	10.3
Bacillus thuringiensis var. *israelensis*	103.24	86.36	118.58	3.5
bendiocarb	5.55	4.83	6.20	5.5
glyphosate	47.31	41.06	51.69	8.9
isostearyl alcohol	>10,000	—	—	—
malathion	49.17	43.26	54.14	9.9
propoxur	1.43	1.19	1.75	2.8
resmethrin + PBO	0.00082	0.00073	0.00093	4.7
thiobencarb	9.24	8.52	10.70	6.9

[1] Body size ranged from 25 to 40 mm.

Table 3. Twenty-four hour probit analyses of toxicities for selected bacterial and chemical pesticides against fourth instar *Anopheles quadrimaculatus* larvae, with 72 hr results for isostearyl alcohol.

Pesticide	LC_{50} (ppm)	95% fiducial limits		Slope
		Lower	Upper	
Bacillus sphaericus	0.021	0.018	0.025	2.2
Bacillus thuringiensis var. *israelensis*	0.014	0.013	0.015	6.9
bendiocarb	0.081	0.069	0.091	4.1
glyphosate	673.43	572.57	770.17	3.2
isostearyl alcohol	0.025	0.018	0.038	1.6
malathion	0.069	0.060	0.080	3.3
propoxur	0.45	0.41	0.51	4.2
resmethrin + PBO	0.0023	0.0018	0.0028	2.4
thiobencarb	3.91	3.36	4.15	4.9

Table 4. Twenty-four hour probit analyses of toxicities for selected bacterial and chemical pesticides against fourth instar *Culex salinarius* larvae, with 72 hr results for isostearyl alcohol.

Pesticide	LC_{50} (ppm)	95% fiducial limits Lower	Upper	Slope
Bacillus sphaericus	0.037	0.030	0.043	3.0
Bacillus thuringiensis var. *israelensis*	0.0062	0.0055	0.0072	4.4
bendiocarb	0.15	0.11	0.18	2.3
glyphosate	1,563.69	1,262.00	2,214.54	2.1
isostearyl alcohol	0.81	0.76	0.86	8.7
malathion	0.053	0.047	0.061	4.1
propoxur	0.18	0.16	0.19	5.0
resmethrin + PBO	0.012	0.0093	0.0153	2.0
thiobencarb	5.39	4.66	6.20	3.3

Table 5. Twenty-four hour probit analyses of toxicities for selected bacterial and chemical pesticides against fourth instar *Psorophora columbiae* larvae, with 72 hr results for isostearyl alcohol.

Pesticide	LC_{50} (ppm)	95% fiducial limits Lower	Upper	Slope
Bacillus sphaericus	0.044	0.039	0.049	3.4
Bacillus thuringiensis var. *israelensis*	0.0063	0.0057	0.0070	3.4
bendiocarb	0.23	0.18	0.31	1.8
glyphosate	940.84	823.08	1,067.12	3.4
isostearyl alcohol	0.32	0.76	0.87	8.6
malathion	0.011	0.10	0.012	5.5
propoxur	0.32	0.29	0.36	5.3
resmethrin + PBO	0.0056	0.0039	0.0063	5.6
thiobencarb	5.72	3.80	6.02	5.0

Table 6. Selectivity ratios of selected pesticides for mosquitoes vs. crawfish.

Pesticide	Selectivity ratio
Bacillus sphaericus	1,708.90
Bacillus thuringiensis var. *israelensis*	7,374.30
bendiocarb	24.10
glyphosate	0.03
isostearyl alcohol	>12,195.00
malathion	712.60
propoxur	3.20
resmethrin + PBO	0.07
thiobencarb	1.60

by dividing the LC_{50} value for crawfish by the largest LC_{50} value obtained for any one of the three mosquito species. Isostearyl alcohol was the most selective pesticide tested, having at least 12,195 times greater toxicity to mosquito larvae than to crawfish. The bacterial pesticides, *B. t. israelensis* and *B. sphaericus*, were the next most selective, with ratios of 7,374 and 1,709, respectively. Malathion was 712 times more toxic to mosquito larvae than to crawfish at the LC_{50} rate. The herbicide thiobencarb showed little if any selectivity, with a ratio of 1.6. The herbicide glyphosate and the insecticide resmethrin + PBO was more toxic to crawfish than larval mosquitoes, with selectivity ratios of 0.03 and 0.07, respectively. However, the so called 'inert ingredients' present in the pesticide formulations may have an impact on these selectivity ratios.

The results of this study should be useful to individuals interested in large-scale commercial crawfish production as well as mosquito control personnel operating in crawfish production areas. The LC_{50} values for crawfish should serve as a guide to pesticide applicators who operate in close proximity to these fields and are concerned about pesticide drift. Comparison of the LC_{50} values between mosquitoes and crawfish indicate that some pesticides may be safely applied near crawfish fields for treatment of larval and adult mosquito populations. In addition, documenting LC_{50} values for pestiferous mosquito species for selected pesticides is important should resistance later be suspected. These LC_{50} values were obtained in the laboratory and may vary according to actual field conditions. Environmental parameters, such as temperature, rainfall, light intensity, physiochemical factors of the habitat (including the mode of pesticide application) may affect the toxicity of the pesticide. The toxicological data provided herein serve as a guide to the relative toxicities of these compounds to the organisms tested.

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