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Response of *Procambarid* Crayfish Populations to Permethrin Applications in Earthen Ponds

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The white river (Procambarus zonangulus formerly P. acutus acutus) and red swamp crayfish (P. clarkii) are two economically important freshwater crustaceans. These species are commercially propagated in areas of intense agricultural activity and wild populations inhabit most of the drainage basins in the southeastern United States (Huner and Barr 1991). In addition to their economic importance and wide geographic distribution, P. zonangulus and P. clarkii possess other characteristics desirable in an aquatic indicator organism. Both species have a small home range, short reproductive cycle, and appear sensitive to a wide range of xenobiotics (Huner and Barr 1991).

Permethrin [(3-phenoxybenzyl (+) cis, trans, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate)] is a broad spectrum synthetic pyrethroid insecticide. The compound has low toxicity to avian and mammalian species (Abernathy and Casida 1973) but is highly lethal to fish (Kumaraguru and Beamish 1981) and crayfish (Jolly et al. 1977; Jarboe and Romaire 1991).

Research examining the impacts of permethrin on procambarid crayfish has included laboratory static acute toxicity determinations (Jolly et al. 1977; Jarboe and Romaire 1991) and mortality observations among caged crayfish in earthen ponds following field applications. The toxicological effects of permethrin on wild populations of procambarid crayfish has not been reported. The purpose of this research was to examine the effects of a permethrin application on naturally reproducing populations of procambarid crayfish in earthen ponds.

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MATERIALS AND METHODS

Earthen ponds (0.044 ha surface area) were prepared for rice production according to standard practices. Ammonium nitrate was disked into pond bottoms at 24.6 kg N/ha, 2 wk prior to rice seeding. Well water was added to ponds to an average depth of 8 cm. Pre-sprouted rice (Oryza sativa, var. Newbonnet) was water-seeded at a rate of 172 kg/ha. Rice was top-dressed with ammonium nitrate (30.9 kg N/ha) at panicle initiation. Ponds were flooded with aerated well water to a depth of 0.5 m in late October. No pesticides were used during any stage of rice cultivation. Dissolved oxygen (DO) and water temperature were measured in each pond daily between 0600 and 0700 hr with a YSI Model 57 oxygen meter (Yellow Springs Instrument Co.).

Experimental ponds contained reproducing populations of P. zonangulus and P. clarkii from previous studies conducted at the experiment station. To insure adequate population size, P. clarkii broodstock adults(1:1 male:female ratio) were stocked into ponds at a rate of 171 kg/ha in late June prior to draining and rice propagation. Broodstock were obtained from other experimental ponds which had no previous history of pesticide treatments.

Crayfish population densities were determined using area-density sampling for small crayfish less than 40 mm total length (TL - tip of rostrum to apex of telson) and the Schnabel mark-recapture procedure for larger crayfish (Everhart et al. 1976). Area-density estimates were obtained by visually subdividing the ponds into 0.5 m^2 quadrats. Six quadrats, selected at random, were sampled by sweeping a dip-net (2.0 mm diamond mesh) along the pond bottom. The number of crayfish captured per dip-net sweep was averaged and extrapolated to total area of the pond. Larger crayfish were captured in baited small-mesh (2.0 mm square mesh) traps. At least 50 mature and intermolt crayfish were marked on the carapace with water insoluble nail polish and released. The numbers of marked and unmarked crayfish in trap samples were enumerated and the process repeated every 2 d over a 9-d period. Molting was considered to be negligible during the sampling period. Population size of crayfish in each pond was determined by adding the totals acquired by the two procedures. Population sizes were determined immediately prior to and 7 d after pesticide application. A final crayfish density estimate was conducted in June. Changes in crayfish population density and composition were compared with nine adjacent ponds which had not been treated with permethrin. It was assumed that any decrease in crayfish number or change in population composition was due to permethrin. Crayfish population profiles (species, sex, stage of maturity, weight and TL) were determined during quantitative population estimates and monthly intervals following pesticide treatment using dip-nets and small-mesh traps. Male crayfish were classified as reproductively active (Form I) or inactive (Form II or juvenile) (Hobbs 1974). Individuals < 15 mm TL were not speciated.

The amount of permethrin required to affect water concentrations ranging from 1-3 μg active ingredient/L was administered to six ponds using a backpack applicator. An 8-L surface composite water sample was collected from several sites throughout the pond immediately prior to application. Total alkalinity, total hardness, pH, total organic carbon (TOC), conductivity, total solids (TS), and biochemical oxygen demand (BOD_5) were determined on the composite water sample. Total alkalinity, total hardness and BOD_5 were determined according to procedures in APHA et al. (1985). The pH was determined with a glass Ag/AgCl pH electrode (Fisher Scientific). Conductivity was measured with a YSI Model 33 conductivity meter (Yellow Springs Instrument Co.). A Model II Technicon Auto Analyzer (Technicon Inc.) was used to quantify TOC. The TS was determined by placing 100 mL of pond water in tared Erlenmeyer flasks and evaporating the samples to dryness in a forced-air convection oven at 100 C for 24 hr. Flasks were then re-weighed and the difference recorded.

RESULTS AND DISCUSSION

Immediately prior to pesticide applications, mean ($\pm$ 1 SD) water temperature, DO, and pH was 19.1(0.4) C, 3.2(1.3) mg/L, and 7.6(0.12), respectively. BOD_5 and TOC averaged 1.7(0.5) mg/L and 5.1(2.2) mg C/L, respectively. Average total alkalinity and hardness was 242(72.7) and 220.7(66.4) mg/L as $CaCO_3$, respectively. Mean TS was 124.8(40.3) mg/L. The number of crayfish captured in small-mesh traps and dip-net samples conducted 7 d post-application indicated that total crayfish population size was reduced 54 to 83% in treated ponds (Table 1). Within the treated pond populations, the decrease among crayfish < 40 mm TL ranged from 55 to 84% and the decrease of crayfish $\geq$ 40 mm TL varied from 42 to 79% (Table 2). Mature male red swamp crayfish experienced

Table 1. Total % mortality of crayfish in earthen ponds as estimated from quantitative population estimates 7 days post-application of permethrin.

Pond	Nominal Permethrin Concentration (μg/L)	% Mortality[1]
1	2.0	83.1
2	2.5	78.6
3	2.0	70.0
4	1.0	54.4
5	2.0	80.4
6	3.0	79.8

[1] - Percent mortality value includes Procambarus clarkii and P. zonangulus.

Table 2. Percent decrease of crayfish < 40 mm total length (TL)and ≥ 40 mm TL as estimated from quantitative population estimates 7 days post-application of permethrin.

Pond	Nominal Permethrin Concentration (μg/L)	%Decrease[1] ≤ 40 mm TL	%Decrease[1] ≥ 40 mm TL
1	2.0	83.8	65.5
2	2.5	80.0	59.1
3	2.0	69.9	78.0
4	1.0	54.6	50.6
5	2.0	80.9	42.2
6	3.0	79.7	79.5

[1] - Percent decrease value includes Procambarus clarkii and P. zonangulus.

the largest decrease of the crayfish exceeding 40 mm TL. A decrease in the number of Form I males in treated ponds ranged from 33 to 100%. Female red swamp crayfish experienced decreases of 43 to 86% and immature male red swamp crayfish declined in numbers from 30 to 72% in treated ponds (Table 3). No significant changes were recorded in crayfish population composition and density in adjacent untreated ponds. Pond populations of P. clarkii appeared to have recovered by the June estimates.

White river crayfish originally comprised 25 and 22% of the crayfish exceeding 40 mm TL in two of the ponds prior to permethrin administration. Following the permethrin applications, no white river crayfish were captured in either of the two ponds. It was assumed that permethrin caused 100% mortality among this species.

Table 3. Percent decrease in mature (Form I) male, immature (Form II) male, and female <u>Procambarus clarkii</u> as estimated from quantitative populations estimates 7 days post-application of permethrin.

Pond	Nominal Permethrin Concentration ($\mu g/L$)	%Decrease Form I	%Decrease Form II	%Decrease Female
1	2.0	64.0	66.0	65.6
2	2.5	33.3	59.1	60.7
3	2.0	100.0	78.0	78.2
4	1.0	46.1	42.9	55.4
5	2.0	68.7	29.8	42.7
6	3.0	88.5	38.9	86.3

In laboratory acute toxicity determinations it has been established that permethrin is lethal to <u>P. clarkii</u> at low concentrations. Jolly and Avault (1978) determined the 96-hr LC_{50} for 8-12 mm TL and 20-30 mm TL juvenile <u>P. clarkii</u> to be 0.39 and 0.62 $\mu g/L$, respectively. Coulon (1982) estimated the 24-hr LC_{50} of juvenile <u>P. clarkii</u> (18-27 mm TL) to be 0.49 $\mu g/L$. <u>P. clarkii</u> that were 8-10, 25-35, 45-55 and 65-75 mm TL had 96-hr LC_{50}'s of 0.44, 0.85, 1.34, and 0.83 $\mu g/L$, respectively (Jarboe and Romaire 1991). Under the conditions of the present study, concentrations of permethrin ranging from 1.0 to 3.0 $\mu g/L$ appear to be acutely toxic to resident earthen pond populations of <u>P. clarkii</u> and <u>P. zonangulus</u>. Comparatively, permethrin ranks as one of the most toxic insecticides to procambarid crayfish in field conditions (Hendrick and Everett 1965; Hendrick et al. 1966; Chang and Lange 1967; Hyde et al. 1972; Ekanem 1981; France 1986).

Mortality among crayfish in ponds indicated that the organism did not respond to permethrin in a typical dose-dependent manner. Organic matter (as measured by BOD_5 and TOC) varied between ponds and may have bound permethrin rendering the compound unavailable for uptake (Ortego and Benson 1992). At the time of permethrin applications, ponds were overpopulated with crayfish relative to forage resources. Physiological stressors resulting from nutritional deficiency and overpopulation may have caused the crayfish to respond to the pesticide exposure in an unpredictable manner. Permethrin toxicity corresponding to size and maturity stage exhibited by <u>P. clarkii</u> in ponds documented by the present study are supported by laboratory observations. Jolly and Avault (1978) and Jarboe and Romaire (1991) reported that newly-hatched and small juvenile <u>P. clarkii</u> were more sensitive to permethrin than larger juveniles of the species. Jarboe and Romaire (1991) demonstrated that permethrin was more toxic to mature male P. clarkii than either female or juvenile males of the same size.

Results of the present study indicate that permethrin is highly toxic to red swamp and white river crayfish at concentrations ranging from 1.0 to 3.0 $\mu g/L$ when applied to earthen ponds. Mortality trends within crayfish populations indicates that permethrin toxicity to P. clarkii can be influenced by size, sex, and stage of maturity.

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