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Effects of the Herbicide Alachlor on Larval Development of the Mud Crab, *Rhithropanopeus harrisii* (Gould)

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ABSTRACT: The effects of the herbicide alachlor, in both technical grade and commercial product form (Lasso), were tested for acute toxicity on larvae of the estuarine crab *Rhithropanopeus harrisii*. The generalized effect is a reduction in survival and a lengthening of developmental time with an increase in concentration. The LC_{50} values were inversely proportional to exposure time and ranged from 10 to 27 mg l^{-1}. Lasso was slightly more toxic than technical grade alachlor.

Introduction

Alachlor [2-chloro-2′-6′-diethyl-N-(methoxymethyl)acetanilide] is the active ingredient (45.1%) in Lasso, a widely used herbicide produced by the Monsanto Agricultural Products Company. The herbicide is used primarily with corn and soybean crops as a preemergent inhibitor of annual grasses, broadleaf weeds, and yellow nutsedge (Monsanto 1984).

Due to its high rate of application (1.68–4.48 kg ha^{-1}), high solubility in water (242 mg l^{-1}) and high stability, alachlor is persistent in soil and aquatic environments (Weed Science Society of America 1979). Detectable levels of alachlor can persist in soils for up to one year and in farm drainage water for up to four weeks (Skaggs et al. 1980). Because of these characteristics, alachlor can readily leach through soils during heavy rainfall. Although most concentrations of alachlor range between 0.078 and 0.184 mg l^{-1} in drainage streams (Wauchope 1978), Skaggs et al. (1980) found levels in farm ditch water as high as 2.7 mg l^{-1} immediately following a runoff event.

Much of the coastal plain wetlands that have been converted to agriculture border directly on ecologically sensitive and economically important estuarine water systems. These systems are susceptible to direct runoff from wetlands agriculture. In recent years, the fates and effects of herbicides and pesticides once they enter the aquatic ecosystem has been of great concern, but little lethal or sublethal toxicity data exist with direct estuarine applicability.

Larvae from the crab *Rhithropanopeus harrisii* were chosen for study because (1) this species is an abundant animal in low salinity headwaters of estuaries, (2) the technique for larval rearing is well known (Costlow et al. 1966), (3) the larval stages are typically the most sensitive to environmental variables (Thorson 1964), and (4) *R. harrisii* larvae have been shown to be sensitive to various pollutants (e.g., Christiansen and Costlow 1975; Forward and Costlow 1978; Bookhout et al. 1980).

In this paper toxicity tests and analysis for posthatch exposure are described for both Lasso alachlor and technical grade alachlor.

Methods and Materials

Preparation of Toxicant and Dilutions

Lasso, an emulsifiable concentrate (EC) of alachlor, was obtained commercially, while the technical grade alachlor was supplied in crystalline form by the Monsanto Agricultural Products Co., St. Louis, Missouri. For experiments on the effects of alachlor, a stock solution was made from each form of alachlor. Ten ml of Lasso EC alachlor (480 g l^{-1}) was pipetted into a glass 1 l bottle and allowed to evaporate. Certified A.C.S. acetone was then added to give a final volume of 480 ml stock solution, which produced a nominal concentration of 10 mg ml^{-1} (10,000 mg l^{-1}). Stock solution of the technical grade alachlor crystal was created by dissolving 3 g of the crystal in 300 ml of acetone, for a stock solution of 10 mg ml^{-1}. Stock solutions were stored in the dark at 5 °C and were replaced after each 30-day period.

Standard dilution techniques were used for both solutions. Acetone serial dilutions of 2,500, 1,250, 500, 50, and 5 mg l^{-1} were made daily. To each 7-cm diameter glass finger bowl, 1 ml of dilution

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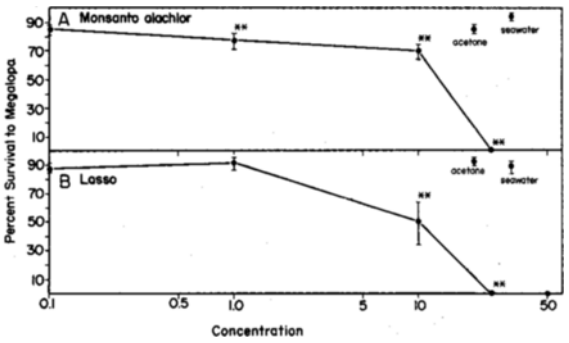

Fig. 1. The percent of larvae developing to the megalopa stage plotted against concentration (mg l^{-1}) of Monsanto alachlor (A) and Lasso (B). Means and standard errors are shown. The n for each determination was 15. Acetone and seawater indicate the percent survival in the acetone and seawater control solutions. The asterisk indicates mean percent development is significantly ($p < 0.05$) different from the seawater control.

was pipetted and allowed to evaporate in a hood. The remaining alachlor film was then resuspended in 50 ml of filtered (5 μm) seawater (salinity 20‰) to give final alachlor concentrations of 50, 25, 10, 1, and 0.1 mg l^{-1}. Acetone controls and seawater controls were also tested. Acetone controls were set up by pipetting 1 ml of acetone into each test bowl and allowing it to evaporate. This was designed to reveal any possible effects of an acetone residue following evaporation on the test bowls. The additional control was to expose larvae to filtered (5 μm) seawater (20‰).

All glassware to be used in herbicide toxicity tests was first washed in a 10% HCl bath, and then washed in a concentrated (37 N) H_2SO_4 bath, followed by a deionized water rinse and a final acetone rinse. Subsequent daily washes consisted of scrubbing bowls with a clean brush in deionized water, followed by an acetone rinse. Separate bowls were used at each concentration throughout the experiment to reduce the risk of cross contamination among concentrations.

Rearing of Larvae

Ovigerous females of *Rhithropanopeus harrisii* (Gould) were collected from the Neuse River in North Carolina. Females were held individually in an environmental chamber in large (19 cm diameter) finger bowls containing 20‰ filtered (5 μm) seawater. Chamber temperature remained at 25 °C (±1 °C), and a 12:12 LD cycle was maintained. Hatching normally occurred 2–3 h after the dark phase began (Forward et al. 1982).

Upon hatching, the adult female was removed from the bowl, and larvae were fed newly hatched *Artemia* sp. *nauplii*. Larvae of *R. harrisii* were also reared under the same environmental conditions as above (20‰; 25 °C; and a 12:12 LD cycle). These parameters have been shown to be optimum conditions for laboratory rearing and development in *R. harrisii* larvae (Costlow et al. 1966).

Approximately 12 h after hatching, larvae from each of three hatches (minimum hatch size: 400 larvae) were placed in the test solution. Five replicates of 10 larvae from each hatch were tested in each solution. Thus the total number of replicates for each condition was 15. Dilution and control solutions were renewed daily, and larvae were fed newly hatched *Artemia* sp. *nauplii*. Dead larvae and molts were counted and removed at this time.

Toxicity Experiments

Three experimental series were conducted. First, the chronic tests consisted of continuously exposing larvae to the test solution throughout larval development. Effects were evaluated as percent survival at the megalopa stage and time duration to reach this stage. Both technical alachlor and Lasso were tested.

In the second experiment larvae were exposed to test solutions continuously for 96 h beginning just after hatching. Survival after this time was recorded. This experiment used both technical alachlor and Lasso and was designed to determine the 96-h mean lethal concentration (LC_{50}).

Finally, the third experiment involved short-term exposure and was designed to test the effect of a simulated short duration runoff event. Larvae were exposed to test solutions of Lasso for 12 h just after hatching during the light phase and then transferred to clean seawater. Mortality was measured after 96 h.

For data analysis, mean percent survival and the standard errors were calculated after arcsine transformation of the data. Since all experiments consisted of a control and many test concentrations, differences were tested using a Dunnett *t* test for multiple comparisons with a control (Dunnett 1964). The LC_{50} values were determined by probit analysis.

Results

Chronic Exposure to Monsanto Alachlor and Lasso

When exposed to 25 and 50 mg l^{-1} of Monsanto alachlor (Fig. 1A), no larvae completed development to the megalopa stage. At concentrations of 1 and 10 mg l^{-1}, survival was significantly lower than in seawater alone; whereas, at 0.1 mg l^{-1} and in the acetone control, survival was similar to that in seawater (Fig. 1A). The LC_{50} level was 14 mg l^{-1}. For larvae that survived, the duration of larval

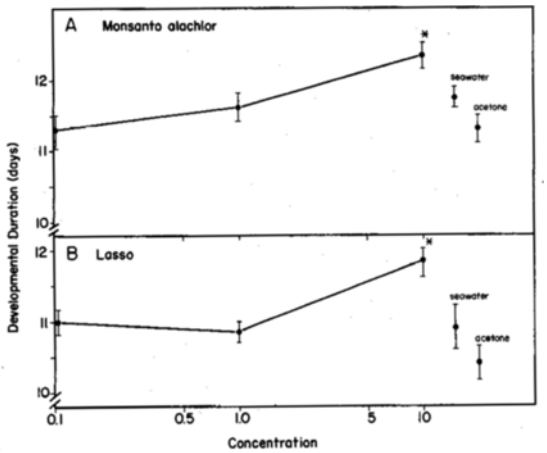

Fig. 2. The number of days for development from stage I zoea to megalopa plotted against concentration (mg l^{-1}) of Monsanto alachlor (A) and Lasso (B). Mean and standard error times are shown and the average n was 14 in A and 11 in B. Seawater and acetone indicate developmental times in these control solutions. Double asterisks indicate development time is significantly ($p < 0.01$) longer than in seawater.

development to the megalopa stage increased with Monsanto alachlor concentration and was significantly longer than that in seawater at 10 mg l^{-1} (Fig. 2A).

Similar results were obtained with Lasso. No larvae survived at 25 and 50 mg l^{-1}, and the lowest test concentration to significantly reduce survival was 10 mg l^{-1} (Fig. 1B). Survival in all other test conditions was not significantly different from levels in seawater. The LC_{50} was 10 mg l^{-1}. The length of larval development increased as survival decreased and was significantly longer at 10 mg l^{-1} (Fig. 2B).

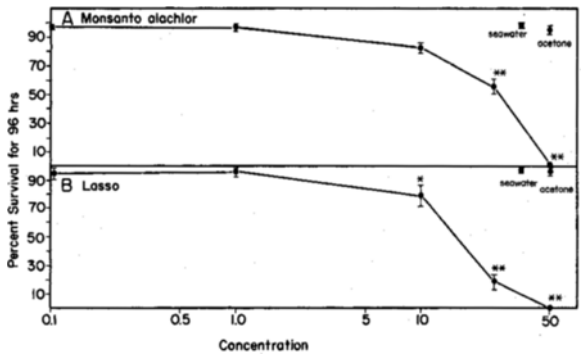

Fig. 3. The percent of larvae surviving for 96 h after exposure to various concentrations (mg l^{-1}) of Monsanto alachlor (A) and Lasso (B). Means and standard errors are plotted. The n for each determination was 15. Seawater and acetone indicate the percent survival in these control solutions. The single and double asterisks indicate survival was significantly lower than levels in the seawater control at the $p < 0.05$ and 0.01 levels, respectively.

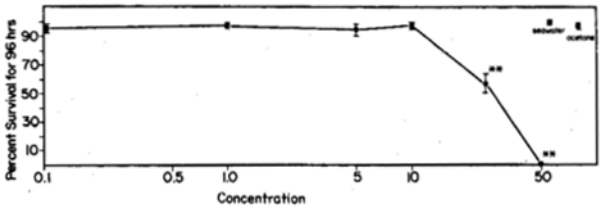

Fig. 4. The percentage of larvae surviving after 12-h exposure to various concentrations (mg l^{-1}) of Lasso. Means and standard errors are plotted and the n for each concentration was 15. Seawater and acetone indicate percent survival in these control solutions. Double asterisks show those concentrations at which the percent survival is significantly lower than that in the seawater control at the $p < 0.01$ level.

Acute Exposure for 96 h to Monsanto Alachlor and Lasso

Exposing larvae to the herbicide for the first 96 h of development also caused a significant reduction in survival. In Monsanto alachlor significant effects were seen at 25 and 50 mg l^{-1} (Fig. 3A). The LC_{50} was 26 mg l^{-1}. Larvae were more sensitive to Lasso, the lowest concentration to significantly lower survivorship was 10 mg l^{-1} (Fig. 3B). The Lasso LC_{50} occurred at 16 mg l^{-1}, a lower concentration than alachlor.

Acute Exposure for 12 h to Lasso

As larvae were more sensitive to short-term exposure to Lasso (Fig. 3B), larvae were exposed to various concentrations for 12 h and then placed in clean seawater. Mortality was determined after a total time of 96 h. Larval survival was significantly reduced at concentrations of 25 mg l^{-1} and greater (Fig. 4). The LC_{50} was 27 mg l^{-1}.

Discussion

The generalized effect of alachlor on *Rhithropanopeus harrisii* larvae is a reduction in survival and a lengthening of developmental time with an increase in concentration. Sensitivity was inversely proportional to exposure time; the estimated LC_{50} concentrations decreased as exposure time increased (Table 1).

The usefulness of larval crustaceans as test organisms for pollutants entering estuarine systems

TABLE 1. The estimated LC_{50} values upon exposure to Monsanto alachlor and Lasso for various time periods.

	LC_{50}	
Exposure Time	Monsanto alachlor mg l^{-1}	Lasso mg l^{-1}
Continuous (Fig. 1)	14	10
96 h (Fig. 3)	26	16
12 h (Fig. 4)	—	27

has been discussed in detail by Epifanio (1979). The planktonic larval stages of crustaceans are considered the most sensitive to environmental perturbations in the life cycle (Thorson 1964). That larval crustaceans usually are much more sensitive to pesticides than adults has been dramatically illustrated by Wilson (1985), who found that grass shrimp (*Palaemonetes pugio*) had a 96-h LC_{50} for diflubenzuron (an insect growth regulator) of 1.4 $\mu g\ l^{-1}$ as larvae, 1.6 $\mu g\ l^{-1}$ as postlarvae, 202 $\mu g\ l^{-1}$ as males and nonovigerous females, and 6,985 as ovigerous females.

The LC_{50} concentrations for *R. harrisii* larvae range from 10 to 27 mg l^{-1} alachlor (Table 1). These values are similar to those for an adult nonmarine crustacean (crayfish), where the LC_{50} for alachlor was 19.5 mg l^{-1} (Weed Science Society of America 1979). The LC_{50} concentrations, however, vary with the type of alachlor tested. Lasso EC alachlor, with its inert detergent carriers, is slightly more toxic (lower LC_{50} values) to *R. harrisii* larvae than technical grade Monsanto alachlor (Table 1). This result is in partial agreement with past studies. Acute dermal LD_{50} studies on rabbits show Lasso to be more toxic than technical grade alachlor (Weed Science Society of America 1979). However, freshwater fish (bluegill, sunfish, trout) were more sensitive to technical grade alachlor than Lasso.

In field studies the alachlor concentration in water of ditches draining a coastal plains farm in North Carolina peaked at times of peak flow and usually ranged up to approximately 0.07 mg l^{-1} (Skaggs et al. 1980). These concentrations are well below those causing mortality in crab larvae. However, on several occasions Skaggs et al. (1980) reported concentrations up to 2.7 mg l^{-1}, values much closer to LC_{50} levels. Although there were no data available, they suggested that these concentrations may have resulted from wind drift spray falling directly into the ditches. Thus there is the possibility of rare-to-occasional short-term exposure to significant concentrations of alachlor by crab larvae in upper estuarine creeks which receive direct drainage from farms. The 12-h pulse experiments simulated a heavy runoff event where concentrations of the herbicide would be at high levels for a short period of time. *R. harrisii* adults inhabit low salinity areas in creeks, thus they are in a position to be exposed to undiluted runoff water. Since early stage larvae are sensitive to short-term exposure (12 h), reproductive success could be affected by alachlor.

Even though the present study indicates *R. harrisii* larvae are sensitive to alachlor, it is possible that even greater sensitivity exists during embryo development. Thus future experiments will consider what effect exposing the eggs to alachlor has on future larval survival. In addition, sublethal effects on larval behavior should be investigated as these might indicate ecological effects which could influence the crabs at concentrations well below that necessary to produce direct mortality.

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