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Responses of *Hyalella azteca* to a Pyrethroid Mixture in a Constructed Wetland

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Constructed wetlands used to mitigate pesticide runoff from agricultural fields into receiving systems (e.g., lakes, rivers, streams) have been successful in reducing concentrations of non-point source pollutants such as agricultural insecticides (Moore et al., 2002; Leistra et al., 2003; Bouldin et al., 2005). Such wetlands also have important functions in enhancing the water quality and ecological values (Moore et al., 2002), and different phases (i.e., aqueous, sediment and detritus) have separate roles, as either sinks or sources, in determining effectiveness of wetlands in mitigating pesticide toxicity. For these reasons, the importance of elucidating potential effectiveness of wetlands in reducing pesticide toxicity to aquatic biota exposed to these different phases needs to be addressed.

The pyrethroid insecticides, λ-cyhalothrin and cyfluthrin, were used as model contaminants in a constructed wetland located in Leflore County, Mississippi, USA, designed to mitigate runoff from an agricultural field. Pyrethroids are used on a variety of agricultural crops in Mississippi, however most applications are primarily with cotton (*Gossypium* sp.) (USDA, 2004). Approximately 3,150 kg of λ-cyhalothrin and 10,350 kg of cyfluthrin were applied in Mississippi in 2003 and, as a result, may contribute to non-point source contamination of aquatic environments (USDA, 2004).

This study examined the use of a constructed wetland to mitigate ecological impacts of a simulated pyrethriod mixture (λ-cyhalothrin and cyfluthrin) in runoff from agricultural fields to receiving aquatic systems by using 48 h aqueous, detrital and sediment bioassays with the freshwater test organism, *Hyalella azteca*.

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Materials and Methods

The constructed wetland used was designed for the mitigation of agricultural contaminant runoff (e.g., sediment, pesticides, and nutrients).

Divided into three cells, the wetland system included a sediment retention pond (SRP), a primary (1°) cell, and a secondary (2°) cell located adjacent to Beasley Lake in Sunflower County, Mississippi, USA. In August 2003, the constructed wetland was amended with 9 ng/mL λ-cyhalothrin (active ingredient (a.i.)) as Karate® and 39 ng/mL cyfluthrin (a.i.) as Baythroid® with 403,000 µg/L sediment (as a carrier) simulating a single 1.3 cm rainfall event and runoff from a 14 ha agricultural field. One liter of water and sediment each, and one leaf litter pack (20 g initial dry weight; simulating detritus) were collected from each wetland cell 1 d, 7 d, 13 d, 27 d, 42 d, and 61 d (water and sediment only) after initial dosing. Samples were preserved on ice and transported the USDA-ARS National Sedimentation Laboratory, Oxford, Mississippi for biological and chemical analysis.

Aqueous, sediment and leaf litter samples were analyzed for λ-cyhalothrin and cyfluthrin. Analytical chemistry was conducted according to Bennett et al. (2000) using a Hewlett-Packard 6890 gas chromatograph equipped with dual HP 7683 ALS autoinjectors. Briefly, aqueous samples were extracted by sonification with reagent-grade KCl and 100 ml pesticide-grade ethyl acetate, dried over anhydrous sodium sulfate, subjected to cleanup by silica gel column chromatography, and concentrated to 1 mL for analysis

(Cooper et al., 2003). Sediment and leaf litter samples were dried, ground, pre-wetted with ultra-pure water followed by the addition of ethyl acetate. The mixture was sonicated and centrifuged (2000–2500 rpm). Extract was concentrated to near dryness using a nitrogen evaporator, and solvent exchanged into hexane. Level of quantification for aqueous, sediment and leaf litter analyses were 0.01 ng/mL, 0.5 ng/g, and 0.5 ng/g, respectively.

Forty-eight hour static, non-renewal, aqueous, sediment and leaf litter toxicity tests using 4–5 d old *Hyalella azteca* were conducted according to modified USEPA (1994) protocol for conducting aqueous reference toxicity tests with a survival endpoint. Aqueous exposures consisted of 12 mL wetland sample water and one, 7 mm diameter, Norway maple (*Acer platanoides*) leaf disc as a substrate and food. One *H. azteca* was placed in each exposure chamber. Similarly, sediment bioassays were conducted as per aqueous tests with 2 g wet wetland sample sediment and 10 mL overlying control water. Leaf litter bioassays were conducted as per aqueous tests with one 7 mm diameter wetland sample leaf disc and 12 mL overlying control water. Overlying control water, free from priority pollutants, was obtained from the University of Mississippi Field Station, filtered to remove particulate matter using MFS 0.45 µm polymembrane filters, and hardness and alkalinity adjusted with $NaHCO_3$ and CaCl to values between 60–80 mg/L as $CaCO_3$ (Deaver and Rodgers, 1996). Toxicity tests were conducted in a Powers Scientific, Inc. Animal Growth Chamber with a 16:8 h photoperiod at 20±1°C. Mean measured physical and chemical water characteristics for aqueous, sediment and leaf litter tests were: temperature, 20.5, 20.5, 20.4°C; pH, 7.6, 6.9, 8; dissolved oxygen, 7, 5.7, 7.3 mg/L; conductivity, 95, 305, 381 µmhos/cm; hardness, 41, 75, 105 mg/L as $CaCO_3$; and alkalinity, 37, 31, 57 mg/L as $CaCO_3$ (APHA, 1998). Sediment characteristics within the constructed wetland were a silt loam (sand 2–45%, silt 52–92%, clay 5–8%) with 1–5% total organic carbon.

H. azteca 48 h aqueous, sediment and leaf litter survival data were analyzed using a Kruskal-Wallis one-way ANOVA on ranks (survival x time within wetland cell) with Dunn's multiple range test versus controls (0 d pre-dosing). Data analysis was conducted using SigmaStat® v.2.03 statistical software (SPSS, 1997).

Results and Discussion

Chemical analysis revealed spatial and temporal variation in λ-cyhalothrin and cyfluthrin concentrations within aqueous, sediment, and leaf litter samples among all three wetland cells (Fig. 1). All wetland cells (SRP, 1° cell, 2° cell) had measurable amounts of cyfluthrin within all three phases 1 d after amendment, but λ-cyhalothrin was only sporadically detected in either 1° or 2° wetland cells. Aqueous and leaf litter pyrethroid concentrations typically decreased with increasing time periods due, in part, to material degradation and desorption. By 61 d, there was little to no measurable amounts of either λ-cyhalothrin or cyfluthrin in either aqueous or leaf litter phases. Spatially, lowest aqueous and leaf litter pyrethroid concentrations occurred within the wetland area furthest from the injection point, the 2° wetland cell. Greatest aqueous and leaf litter concentrations occurred within the sediment retention pond. Spatial and temporal patterns of sediment pyrethroid contamination were near inverse characteristics. Such a pattern shows pooling of water and associated pesticide within the sediment retention pond and continued slow movement into the remaining two cells. Similar spatial and temporal patterns of transfer/transformation were observed by Leistra et al. (2003) for λ-cyhalothrin in ditch systems within aqueous, sediment and plant phases. However, Bouldin et al. (2005) did not show similar patterns in sediment λ-cyhalothrin contamination due, in part, to lower initial dosage and static aqueous conditions within their microcosms.

Hyalella azteca 48 h survival in aqueous exposures varied temporally in conjunction with measured pyrethroid concentrations (Fig. 1). Limited pretreatment (time 0 h) aqueous survival (< 80%) occurred due to the very soft nature of the natural wetland water. Aqueous hardness and alkalinity ranged from 17.1–51.3 mg/L as $CaCO_3$ and 34.2–51.3 mg/L as $CaCO_3$, respectively, resulting in less than optimal survival rates. Grapentine and Rosenberg (1992) observed *H. azteca* to be in low abundance or absent from lakes with lower calcium concentrations (< 2 mg/L). However, clear patterns of exposure effects were observed. Aqueous survival decreased significantly, compared to time 0 d, in all three wetland cells [SRP ($P < 0.001$, H = 17.7); 1^o cell ($P = 0.003$, H = 11.6); 2^o cell ($P < 0.001$, H = 46.0)] and throughout the sampling period after pyrethroid dosing. No decrease in aqueous toxicity occurred during the 61 d observation period despite significant decreases in pyrethroid concentrations. Lowest observed effect concentration for the λ-cyhalothrin and cyfluthrin mixture was 0.07 and 0.22 ng/mL. Cyfluthrin (only) lowest observed effect concentration was 0.05 ng/mL. Reported aqueous λ-cyhalothrin and cyfluthrin effects concentrations for crustaceans are at < 0.2 ng/mL, which is approaching the lowest observed aqueous pyrethroid concentration (0.05 ng cyfluthrin/mL). Maund et al. (1998) reported *H. azteca* 48 h aqueous λ-cyhalothrin EC50 of 0.0023 ng/mL. Reported cyfluthrin EC50s were 0.012, 0.14, and 0.17 ng/mL for saltwater mysid shrimp (*Americamysis bahia*), freshwater *Daphnia magna*, and *Ceriodaphnia dubia*, respectively (Mokry and Hoagland, 1990; Solomon et al., 2001).

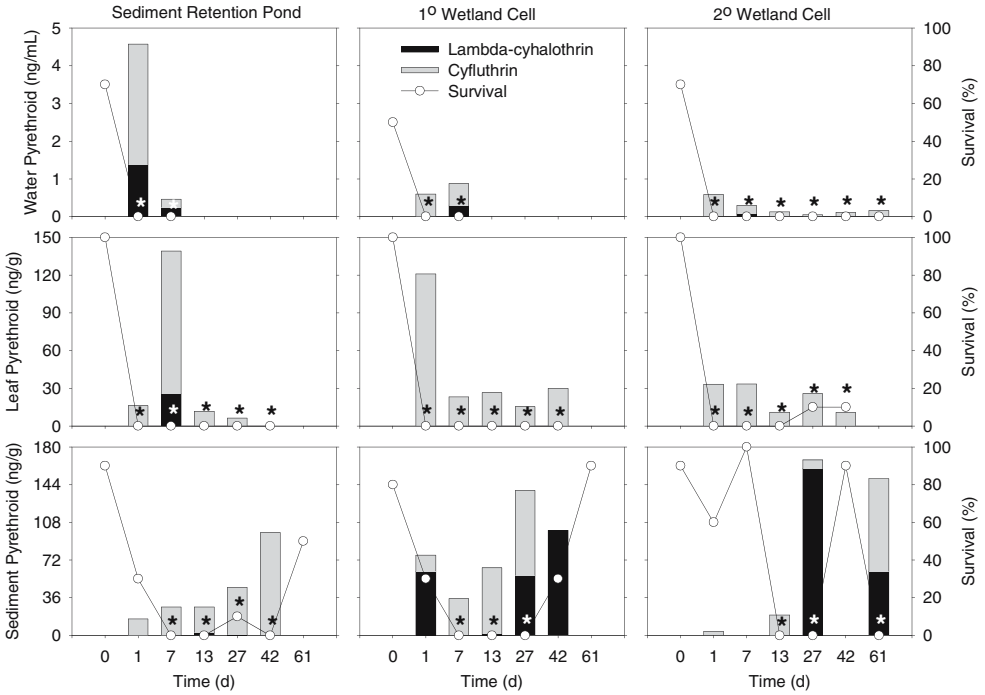

Fig. 1 Responses of *Hyalella azteca* 48 h survival exposed to lambda-cyhalothrin and cyfluthrin in water, leaf litter, and sediment phases in a constructed wetland. Asterisks indicate significantly different temporal survival ($P < 0.05$)

Pyrethroid contaminated leaf litter elicited similar *Hyalella azteca* toxicity responses, however with greater effects concentrations (Fig. 1). Survival decreased significantly, compared to time 0 d, in all three wetland cells [SRP ($P < 0.001$, H = 59.0); 1° cell ($P = 0.003$, H = 59.0); 2° cell ($P < 0.001$, H = 47.9)] and throughout the sampling period, after pyrethroid dosing. Lowest observed effect concentration for the λ-cyhalothrin and cyfluthrin mixture was 25 and 114 ng/g. Cyfluthrin (only) lowest observed effect concentration was 6 ng/g. Only limited research has been done to assess insecticide toxicity within detritus (Odum et al., 1969; Swift et al., 1988; Harrahy et al., 1994). In this study, leaf litter remained toxic throughout the 42 d sampling period, showing a significant contribution to overall pyrethroid toxicity in the system.

H. azteca survival in pyrethroid contaminated sediment also varied temporally in conjunction with measured pyrethroid concentrations (Fig. 1), but to a lesser extent than either aqueous or leaf litter exposures, which confirms that sediment bound pyrethroids are less bioavailable than aqueous or detritus pyrethroids. Maund et al. (1998) reported *Chironomus riparius* 28 d sediment λ-cyhalothrin EC50 of 6.8 ng/g organic carbon (oc) whereas Weston et al. (2004) reported estimated *C. tentans* 10 d EC50 of 1.3 ng/g oc. Our results showed the lowest observed effect concentration for the λ-cyhalothrin and cyfluthrin mixture was 3 and 24 ng/g, and cyfluthrin (only) lowest observed effect concentration was 16 ng/g. Patterns of animal survival were similar in SRP and the 1° wetland cell. The 2° cell showed a significant ($P < 0.001$, H = 52.4) decrease in survival 13 d after dosing; however, by 42 d, survival was greater than 80%. Although the 1° cell had relatively high measured sediment pyrethroid concentrations 1 d and 42 d after dosing, relatively lower toxicity is associated with greater total organic carbon (TOC) in these sediments. Sediment from SRP had only 1–1.5% TOC, 1° cell had 2–5% TOC and 2° cell had 1.9–2% TOC. The influence of TOC on the bioavailability of insecticides with low water solubility to *Hyalella azteca* has been previously documented (Nebeker et al., 1989; Amweg et al., 2005). Thus the greater TOC in the 1° cell sediment mitigated the greater pyrethroid concentrations. Again, spatial *H. azteca* responses coincided with measured pyrethroid concentrations, as seen temporally with exceptions associated with differences in sediment TOC.

Based upon responses of *Hyalella azteca* to aqueous, sediment, and leaf litter λ-cyhalothrin and cyfluthrin contamination, sediment and detrital bound pyrethroids can move from contaminant sink during initial pesticide influx to a source of pyrethroid contamination affecting non target aquatic organisms for weeks to months after entering a constructed wetland. Further studies are needed to elucidate the relationship between aqueous, sediment and detrital phases in pesticide contamination within aquatic systems, and the associated effects on non target aquatic organisms.

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