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Hydroponic uptake of atrazine and lambda-cyhalothrin in *Juncus effusus* and *Ludwigia peploides* ☆

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Abstract

Phytoremediation encompasses an array of plant-associated processes known to mitigate contaminants from soil, sediment, and water. Modification of pesticides associated with agricultural runoff includes processes directly associated with aquatic macrophytes in addition to changes in soil geochemistry and associated rhizospheric degradation. Remediation attributes of two vegetative species common to agricultural drainages in the Mississippi Delta, USA, were assessed using atrazine and lambda-cyhalothrin. Concentrations used in 8-d hydroponic exposures were calculated using recommended field applications and a 5% runoff model from a 0.65-cm rainfall event on a 2.02-ha field. While greater atrazine uptake was measured in *Juncus effusus*, greater lambda-cyhalothrin uptake occurred in *Ludwigia peploides*. Maximum pesticide uptake was reached within 48 h for each exposure and subsequent translocation of pesticides to upper plant biomass occurred in macrophytes exposed to atrazine. Sequestration of 98.2% of lambda-cyhalothrin in roots of *L. peploides* was measured after 8 d. Translocation of lambda-cyhalothrin in *J. effusus* resulted in 25.4% of pesticide uptake partitioned to upper plant biomass. These individual macrophyte remediation studies measured species- and pesticide-specific uptake rates, indicating that seasonality of pesticide applications and macrophyte emergence might interact strongly to enhance mitigation capabilities in edge-of-field conveyance structures.

Keywords: Phytoremediation; Atrazine; Lambda-cyhalothrin; *Ludwigia peploides*; *Juncus effusus*; Hydroponic

1. Introduction

Plant remediation of soils, sediments, and water is a cost-effective and resource-conservative approach for clean-up of contaminated sites (Susarla et al., 2002). Phytoremediation is an accumulation of plant-associated processes which include biotransformation, phytoaccumulation, phytoextraction, phytovolatilization, and rhizodegradation from enhanced microbial activity in plant rhizospheres (Walton and Anderson, 1990; Mirgain et al., 1993; Susarla et al., 2002) and plant transformation, conjugation, and sequestration are vital tools in waste management (McCutcheon and Schnoor, 2003). Utilization of plants for removal of heavy metals has been investigated since the early 1970s (Kadlec and Knight, 1996; Hawkins et al., 1997; Gillespie et al., 2000), and various research has demonstrated species mortality as well as tolerant vegetation following metal hyperaccumulation (Susarla et al., 2002). In either case, harvest and disposal, followed by replanting were necessary for complete metal removal. More recently, phytoremediation research with heavy metals in aquatic systems has expanded

☆ All programs and services of the US Department of Agriculture are offered on a non-discriminatory basis without regard to race, color, national origin, religion, sex, marital status, or handicap. Mention of a pesticide in this paper does not constitute a recommendation under FIFRA as amended. Names of commercial products are included for the benefit of the reader and do not imply endorsement or preferential treatment by the US Department of Agriculture.

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to include binding capabilities of byproducts of resident vegetative communities such as dissolved organic matter and humic acids (Kim et al., 1999; De Schamphelaere et al., 2004).

Phytoremediation of organic compounds initially involves organic matter sorption determined by physicochemical properties of the compound, surrounding environmental soil conditions, and root morphological characteristics (Cunningham et al., 1997). Due to their large surface area of fibrous roots and intensive soil penetration, terrestrial grasses are most often used in remediation of organic compounds (Günther et al., 1996; Chekol et al., 2002). Successful remediation of polycyclic aromatic hydrocarbons (PAHs) and trinitrotoluene (TNT) have been shown with annual ryegrass (*Lolium multiflorum*), reed (*Phragmites australis*), reed canarygrass (*Phalaris arundinacea*), and switchgrass (*Panicum virgatum*) (Chekol et al., 2002; Lalande et al., 2003; Muratova et al., 2003). Concurrent with transport and detoxification processes of organic compounds within the plant, interactions within soil rhizospheres provide ideal conditions for co-metabolism with symbiotic bacteria and fungi (Zablotowicz and Hoagland, 1999; Susarla et al., 2002; Godsy et al., 2003; Muratova et al., 2003). Simultaneous interactions of the geochemical environment as a result of plant root zones, such as pH and oxidation, aid initial uptake of organics. Subsequent detoxification begins with conjugation in the cytosol, transfer to and temporary storage in vacuoles, followed by slower detoxification and transport to apoplasts, rhizospheres, or atmosphere (Trapp and Karlson, 2001; Schröder and Collins, 2002).

Perhaps due to the emphasis on investigations with grasses, more is known about currently endorsed agricultural best management practices (BMPs) such as vegetated filter strips (VFS) and riparian zones. Investigations have demonstrated herbicide degradation by wetland riparian soils (Stoeckel et al., 1997) and prairie grasses (Belden et al., 2004), but less consideration has been given to in-place aquatic macrophytes and their phytoremediation capabilities.

Phytoremediation processes of aquatic vegetation within wetland and ditch systems are essential components of pesticide mitigation (Fairchild et al., 1994; Moore et al., 2001; Bouldin et al., 2004b). Investigations contrasting unvegetated systems and those with plant communities (Schulz et al., 2003b; Milam et al., 2004), as well as single plant species (Jones and Estes, 1984; Karen et al., 1998; Hand et al., 2001; Lunney et al., 2004; Bouldin et al., 2005), have illustrated the importance of macrophytes in pesticide mitigation. Although low nutrient accumulation and slow biomass production is characteristic of the macrophyte, *Juncus effusus* (Tanner, 1996), atrazine resilience and accumulation in this species supports its use in phytoremediation (Lytle and Lytle, 2002). Conversely, high nitrogen accumulation through rapid biomass production and tolerance to low herbicide concentrations supports *Ludwigia peploides* in phytoremediation of agricultural wastewater (Rejmánková, 1992).

Plant species with known remediation attributes and presence in agricultural conveyance structures have been proposed for further study (Bouldin et al., 2004a, 2005). Systems with indigenous vegetation are being examined for specific processes that contribute to mitigation of pesticides commonly applied in the Mississippi Delta, USA, and will further the understanding of mitigation capabilities in these receiving systems. The selective herbicide, atrazine (2-chloro-4-ethylamino-6-isopropylamino-S-triazine), and the synthetic pyrethroid, lambda-cyhalothrin [(RS)-α-cyano-3-phenoxybenzyl 3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimenthylcyclopropanecarboxylate], are commonly used pesticides in the lower Mississippi Valley, USA (NASS, 2002). Atrazine was registered for use in the United States in 1959 (US EPA, 1994), and its prevalence in surface water is due to relatively high water solubility, moderate environmental persistence (Table 1) and usage exceeding 28 million kg/y (NASS, 2002). As well, concern for atrazine's effect on non-target organisms has required that subsequent risk assessments in aquatic systems account for residence times in static surface and ground water (Solomon et al., 1996; Dodson et al., 1999).

Lambda-cyhalothrin is a frequently used synthetic pyrethroid with an applied rate of 25 000 kg in 2001 (NASS, 2002). Associated physical and chemical properties of synthetic pyrethroids (Table 1) result in perceived low environmental exposure potential due to relatively short residence time in surface waters (Hand et al., 2001). Exceptional hydrophobicity results in rapid partitioning from the water column, low mammalian and avian toxicity, and relatively short environmental half-lives. However, pyrethroid toxicity measured with aquatic invertebrates in laboratory conditions (Maund et al., 2002; Orme and Kegley, 2003) has led to a number of complex ecological risk assessments.

Sediment organic matter and microbial interactions within plant rhizospheres enhance the degradation of pesticides associated with agricultural runoff (Zhou et al., 1995; Ronday et al., 1997; Zablotowicz and Hoagland, 1999; Xia and Ma, 2006). Rice et al. (1997) suggested practical applications of phytoremediation through the construction of wetlands and macrophyte-cultured areas receiving agricultural runoff, but also iterated the need for further research to distinguish between macrophyte degradation

Table 1
Chemical properties of atrazine and lambda-cyhalothrin[a,b]

	Atrazine	Lambda-cyhalothrin
Water solubility (mg/l)	32	0.005
Adsorption coefficient ($\log K_{oc}$)	1.97	3.37
Partition coefficient ($\log K_{ow}$)	2.34	7.00
Hydrolysis half-life (d)	30	233
Aerobic soil half-life (d)	146	62
Anaerobic soil half-life (d)	159	128
Henry's Law constant (M/atm)	4.086E+05	5.629E+03
Vapor pressure (mPa)	4.0E−2	2.0E−4

[a] Orme and Kegley (2003).
[b] USDA ARS pesticide database (2005).

and remediation through associated pathways. Elimination of soil and rhizospheric interactions through hydroponic exposures enables the quantification of direct macrophyte uptake. Measurement of pesticide uptake and partitioning in *J. effusus* and *L. peploides* were achieved in this study through hydroponic laboratory exposures of atrazine and lambda-cyhalothrin.

2. Methods and materials

J. effusus (L) subsp. *solutus* (Fernald & Wiegand) Hämet-Ahti was obtained from a commercial aquatic supplier with guaranteed absence of pesticide exposure. Upon receipt, plants were placed into Rubbermaid™ tubs filled with municipal dechlorinated tapwater (Jonesboro, AR) in a greenhouse environment for 16 d prior to pesticide exposure. Unavailability of *L. peploides* (H.B.K.) Raven. subsp. *glabrescens* (Kuntze) Raven from commercial nurseries necessitated excavation from a local wetland with no history of pesticide applications. These macrophytes were placed in Rubbermaid™ tubs with indigenous sediment and municipal dechlorinated tapwater (Jonesboro, AR) and transferred to a greenhouse environment for 23 d. Equilibration time in a greenhouse environment insured actively growing macrophytes prior to pesticide exposure. Prior to placement into pesticide solution, plant roots and lower stems were thoroughly rinsed with dionized water to dislodge any solid material and insure maximum hydroponic contact. Plant vouchers for each species are available in Arkansas State University's herbarium.

2.1. Treatment application

Pesticide treatments were calculated using the recommended field dose of atrazine (2.23 kg a.i./ha) and lambda-cyhalothrin (0.028 kg a.i./ha) with 5% runoff from a 0.65-cm rainfall event on a 2.02-ha field. Stock solutions (100X) of atrazine as Aatrex® and lambda-cyhalothrin as Karate® were prepared prior to dosing in 1-l glass volumetric flasks. Pre-marked 500-ml Erlenmeyer flasks received a 250-ml dilution of individual pesticide solution prior to macrophyte introduction. Macrophyte roots and extremely lower stem in each flask were exposed to pesticide solution as to mimic contact in agricultural receiving systems.

2.2. Pesticide analyses

Chemical analyses were conducted on hydroponic solution, root washes, roots, and upper stem and leaf areas to determine pesticide concentrations throughout the exposures as described by Bennett et al. (2000) and Smith and Cooper (2004). Atrazine and lambda-cyhalothrin were analyzed via HP 6890 gas chromatograph equipped with a 30-m HP 5MS column. A multi-level calibration procedure was utilized with standards and updated every ninth sample. Limits of detection (LOD) for atrazine in water and plants were 0.01 μg/l and 0.1 μg/kg wet weight, respectively; additionally, the limit of quantitation (LOQ) for atrazine in water was 0.1 μg/l. LOD for lambda-cyhalothrin in water, sediments, and plants were 0.001 μg/l and 0.01 μg/kg wet weight, respectively; additionally, LOQ for lambda-cyhalothrin in water was 0.01 μg/l. Mean extraction efficiencies based on fortified samples, were >90% for water and plants.

2.3. Exposure and sample collection

Aqueous samples were collected prior to macrophyte exposure for background pesticide concentrations. Hydroponic exposure occurred in a Conviron® growth chamber (Model 8507); as to mimic early growing season of May–June, diurnal temperature and light ranged from 18.0–29.8 °C and 7800–32 900 lx, respectively, and relative humidity was maintained at 60–70%. Sample collection included three replicates of *J. effusus* and *L. peploides* exposed to each pesticide solution for 8 h, 24 h, 48 h, 5 d and 8 d. Sample collection included remaining pesticide solution, loosely bound pesticide from exposed roots (adsorbed), macrophyte roots (roots), and remaining stem and leaves (upper biomass). Collection method followed as such: (1) following deionized (DI) water rinse of pesticide-exposed area, adsorbed (loosely bound) pesticide was dislodged with gentle agitation in 100% ethyl acetate (EtOAc), (2) combined rinse water and remaining hydroponic solution were measured and extracted immediately in amber glass jars with the addition of 0.5 mg KCl and 50 ml EtOAc, (3) plant roots were cut with EtOAc-cleaned scissors, weighed, placed in individual airtight plastic containers, and stored at −80 °C prior to extraction, (4) plant stem and leaves were cut into 2–3 cm sections, weighed, and stored as described above. Additional replicates of *J. effusus* and *L. peploides* exposed to DI water lacking pesticide dosages were incubated and collected at 8 d as described above.

Plant extractions were performed with modifications of the method described by Dayan et al. (1997) with mortar and pestle maceration following exposure to liquid nitrogen. Following retrieval from the −80 °C freezer, extraction method followed as such: (1) addition of liquid nitrogen facilitated crushing to a powder, (2) subsequent addition of 100% EtOAc and further crushing to dislodge plant-bound pesticides, (3) transfer of macerated plant/EtOAc mixture to glass centrifuge tubes, (4) 15–20 s vortex followed by 10–15 min extraction at room temperature, (5) centrifugation for 10 min at 2500 rpm, and (6) transfer of EtOAc supernatant into pre-labeled amber glass jars until analyzed. Results were calculated as measured concentrations/kg wet weight.

3. Results

3.1. Atrazine/J. effusus

Atrazine concentrations in hydroponic solutions were below detectable limits throughout the 8-d exposure; however, most atrazine sorbed by the macrophyte was

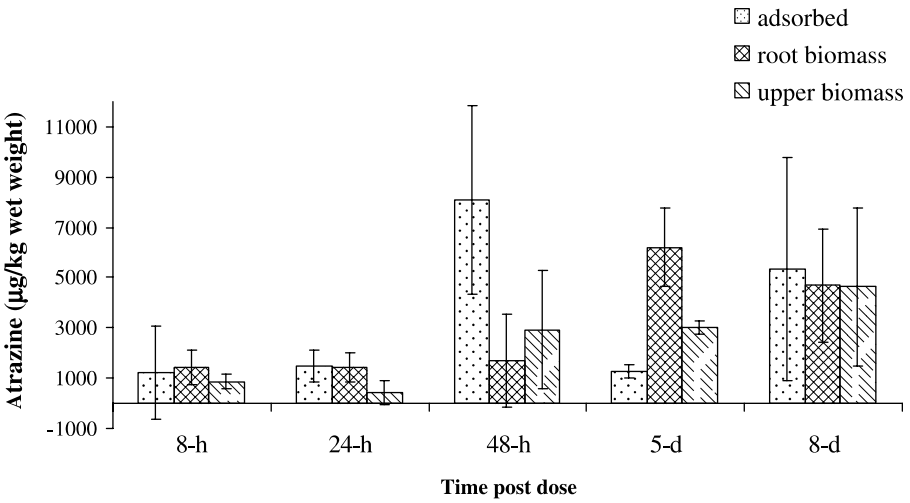

Fig. 1. Atrazine concentrations in *J. effusus* following pesticide exposure. Concentrations are reported as µg/kg wet weight. Means ± 1 SD provided.

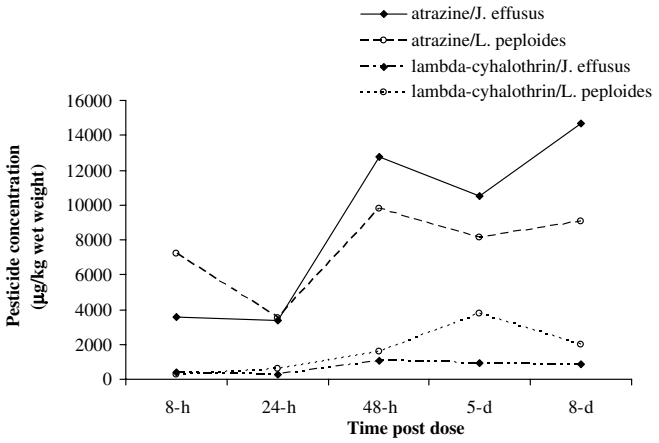

Fig. 2. Total atrazine and lambda-cyhalothrin concentrations in *J. effusus* and *L. peploides* following pesticide exposure. Concentrations are reported as µg/kg wet weight.

concentrated in the roots of *J. effusus* either by adsorption to roots or absorption into root biomass after 8 h and 24 h (75.4% and 86.8%, respectively) (Fig. 1). Pesticide adsorption to roots was highest after 48 h (8093.6 ± 3755.4 µg/kg) and most was absorbed into root biomass after 5 d (6206.2 ± 1559.5 µg/kg). Total atrazine uptake extracted from the hydroponic solution by this macrophyte was highest after 8 d (14697.1 ± 9832.3 µg/kg) with pesticide distributed throughout the plant (adsorbed—37%, roots—32%, upper biomass—32%) (Fig. 2).

3.2. Atrazine/L. peploides

Atrazine uptake by *L. peploides* was distributed throughout the plant after 8 h (adsorbed—30.2%, roots—40.4%, upper biomass—29.4%) (Fig. 3). After 48 h, 50.7% of measurable atrazine had been translocated into the upper biomass (4980.2 ± 1352.6 µg/kg). Atrazine uptake by this

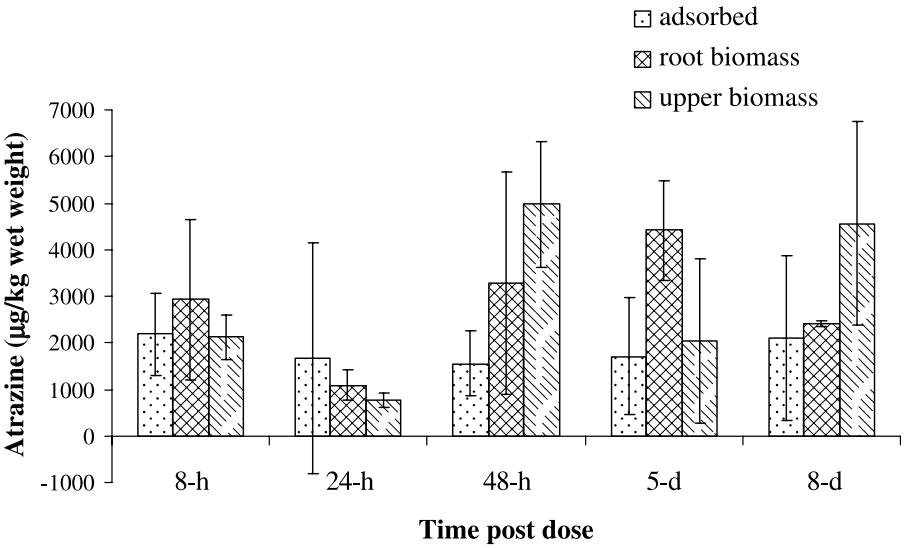

Fig. 3. Atrazine concentrations in *L. peploides* following pesticide exposure. Concentrations are reported as µg/kg wet weight. Means ± 1 SD provided.

macrophyte was greatest at 48 h (9817.1 ± 4423.8 μg/kg) and root uptake at 5 d was followed by translocation to the upper plant biomass after 8 d.

3.3. Lambda-cyhalothrin/*J. effusus*

Most lambda-cyhalothrin (72.1%) adsorbed to *J. effusus* roots within 8 h of exposure with 58.8% remaining adsorbed after 48 h (Fig. 4). Pesticide moved into root tissue by 5 d (548.30 ± 480.79 μg/kg) and remained in this compartment throughout the 8-d exposure (634.33 ± 324.36 μg/kg). Only 25.4% of pesticide translocated to upper plant tissue after 8 d.

3.4. Lambda-cyhalothrin/*L. peploides*

Pesticide concentrations from roots of *L. peploides* included 52.5% absorbed and 45.0% bound in root biomass after 8 h (Fig. 5). After 24 h, 85.5% of pesticide uptake was partitioned in the root biomass and remained there for the 8-d exposure (1913.36 ± 2238.24 μg/kg).

4. Discussion

More rapid atrazine uptake was observed after 8 h in *L. peploides* resulting most likely from reported high macrophyte biomass production (Rejmánková, 1992). Following

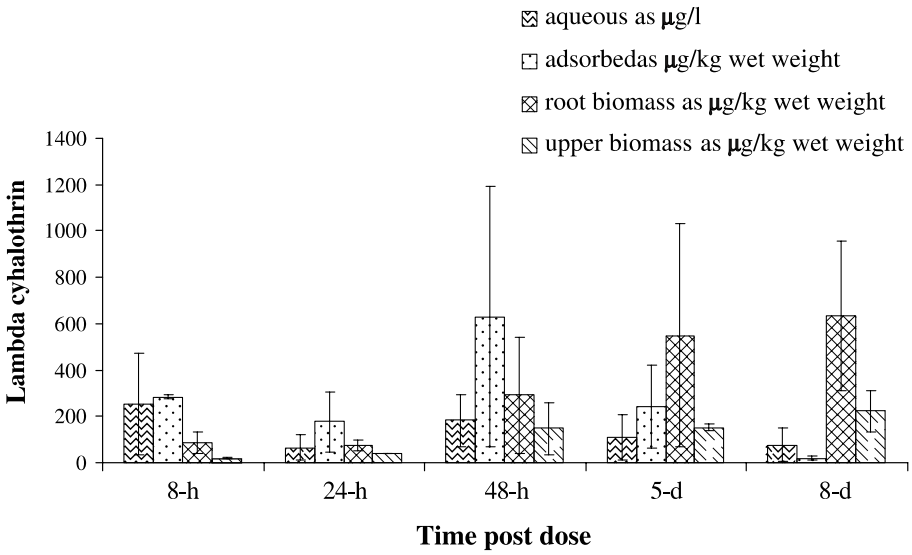

Fig. 4. Lambda-cyhalothrin concentrations in *J. effusus* and remaining hydroponic solution following pesticide exposure. Aqueous and wet weight concentrations appear on graph. Means ± 1 SD provided.

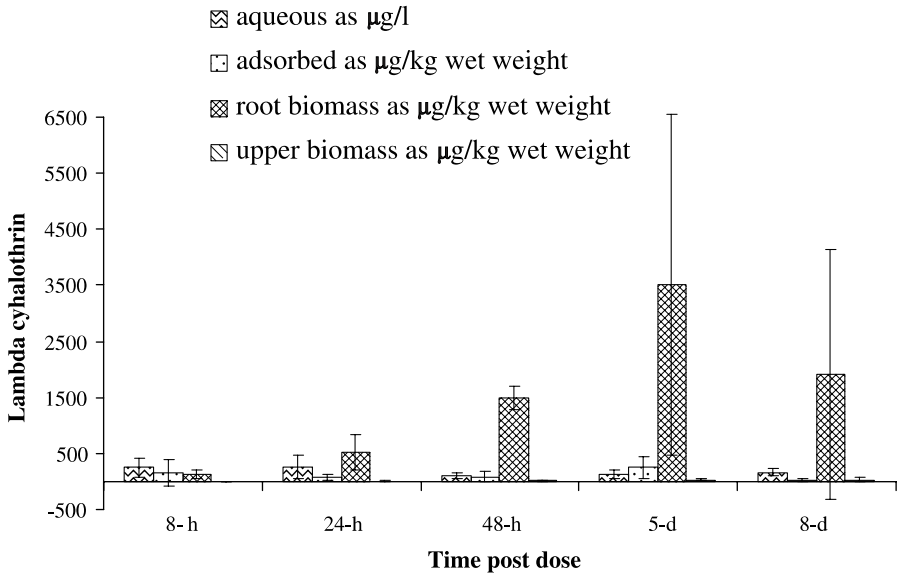

Fig. 5. Lambda-cyhalothrin concentrations in *L. peploides* and remaining hydroponic solution following pesticide exposure. Aqueous and wet weight concentrations appear on graph. Means ± 1 SD provided.

this initial uptake, a second partitioning to root biomass was measured after 5 d. Since translocation into upper biomass preceded these measurements, and mass balance was maintained within the macrophyte, metabolized atrazine may have been atmospherically released or partitioned to apoplastic tissue, allowing for additional uptake. Noticeable stress in *L. peploides* after 5 d did not result in complete senescence of this macrophyte, but may have caused basipetal atrazine translocation at this time.

Although no observable stress was noted in *J. effusus*, lack of new root tissue emergence as observed in control exposures was recorded after 8 d. Such root inhibition may precede observed effects in shoot count and length measurements upon exposure to atrazine (Lytle and Lytle, 2005). Even with less rapid initial atrazine uptake and decreased root growth, herbicide tolerance of *J. effusus* (Lytle and Lytle, 2002) may allow for continued accumulation. In previous microcosm studies rapid atrazine uptake in *L. peploides* was followed with greater accumulation after 7 d (Bouldin et al., 2005), while in the present study, greater atrazine accumulation was measured in *J. effusus* during the 8-d exposure. Measured differences in atrazine accumulation between the two studies may be due to plant material in the previous study retrieved only from the water/plant interphase and may have failed to account for root partitioning or acropetal translocation.

Physicochemical properties of organic compounds dictate absorption and transportation within the macrophyte and compounds with $\log K_{ow}$ 1–3.5 allow mobility and subsequent leaf and stem metabolism (Ashton and Crafts, 1981; Schröder and Collins, 2002). Similar acropetal translocation and observed equilibrium within 6 h of exposure was reported in *Hydrilla verticillata* from atrazine-spiked sediment (Hinman and Klaine, 1992). In the current hydroponic study, equilibrium was accomplished within 48 h, with translocation continuing throughout the 8-d exposure. Observed differences in these studies may have been due to dissimilarity of sediment and hydroponic exposures, submerged and emergent vegetation, or contrasts of 2–3-cm segments of shoot beyond the roots in *H. verticillata* with the entire upper stem and leaves measured in the present hydroponic exposure. Due to slow metabolism of atrazine followed by eventual metabolic release, extended studies may be necessary to demonstrate further uptake by the macrophytes in these studies.

Hinman and Klaine (1992) also reported 96-h equilibrium in *H. verticillata* exposed to chlordane-amended sediment. Uptake and transfer within macrophytes with similar physicochemical properties should allow comparisons of plant accumulation. Variances in plant partitioning were measured as chlordane's (solubility = 0.06 mg/l; $\log K_{ow} = 5.58$) significant translocation into the lower 2–3 cm of the stem (Hinman and Klaine, 1992) contrasted with 74.6% and 98.2% lambda-cyhalothrin partitioning in roots of *J. effusus* and *L. peploides*, respectively. Hydrophobic chemicals tend to be retained in the lipids of the root epidermis and surrounding mucilage (Schröder and Collins, 2002), and significant translocation into stem tissue would not be expected. Variances in uptake and translocation as measured in our study would be expected to be species-specific. This was demonstrated by rapid transfer into root biomass of *L. peploides* which most likely allowed for greater pesticide uptake throughout the 8-d exposure. Translocation of lambda-chyalothrin within macrophytes did not result in greater pesticide uptake. Although *J. effusus* uptake into root biomass was slower and 25.4% was translocated into upper plant biomass, as in previous microcosm studies (Bouldin et al., 2005), greater lambda-cyhalothrin uptake was accomplished in *L. peploides*.

Research utilizing submerged aquatic plants in pesticide phytoremediation exposures (Jones and Estes, 1984; Hinman and Klaine, 1992; Rice et al., 1997; Karen et al., 1998) does not account for nutrient runoff and sediment accretion in agricultural drainage systems shifting macrophyte communities from submerged to emergent vegetation (Phillips et al., 1978; Chambers, 1987; Bhowmik and Adams, 1989; Hough et al., 1989; Janse, 1998). Additionally, recent studies have reported that resident vegetative communities in agricultural drainages of the Mississippi Delta, USA, are composed primarily of emergent species (Bouldin et al., 2004a). Pesticide remediation is enhanced in emergent and floating vegetation by high transpiration rates and lipids associated with plant cuticles (Hutchison, 1975; Williams, 2002; Chefetz, 2003) while greater exposed surface areas enhance remediation in submerged macrophytes (Rice et al., 1997). Increased adsorption onto exposed surface area of submerged macrophytes could be expected, with acropetal translocation of absorbed pesticides impeded by low transpiration rates. In contrast, potential uptake of organic contamination is influenced by evapotranspiration (Cunningham et al., 1997), therefore higher transpiration rates of emergent vegetation could be expected to increase volatilization of pesticide metabolites resulting in greater remediation capabilities.

Investigations of mitigation capabilities within agricultural drainages (Moore et al., 2002; Schulz et al., 2003a; Cooper et al., 2004) fail to distinguish between macrophyte-specific degradation and remediation through associated pathways. In these ecosystems, sediment, organic matter, microbial action, and resident vegetation combine to form a complex dynamic of pesticide remediation (Anderson et al., 1994; Chung et al., 1996; Tanner, 1996; Novak, 1999; Runes et al., 2001). Understanding macrophyte-specific remediation as a component of the dynamic interaction of pesticide mitigation may help further establish optimum vegetative communities within these systems.

Remediation studies of pesticides commonly used in the Mississippi Delta could greatly benefit from encompassing resident vegetation of agricultural receiving systems. Direct macrophyte mitigation of pre-emergent herbicides, such as atrazine, may only be possible through early-season emergent vegetation, such as *J. effusus*, while mid-season applications of pesticides may enter receiving systems with established vegetative communities and maximized mitiga-

tion capabilities. Seasonality of pesticide applications and macrophyte emergence in these systems should be jointly considered in remediation studies. Direct macrophyte contact and phytoremediation processes such as water uptake and volatilization may be determined by seasonality, but indirect mitigation through associated activities such as rhizospheric action continues during plant dormancy (Williams, 2002). Seasonal succession of vegetated communities within agricultural conveyance structures results from varying species emergence and changing hydraulic regime. Such dynamic regimes not only interact to furnish movement through macrophyte systems, but in addition, activate seasonal succession such that ephemeral drainage systems take on specific attributes with regard to plant succession, hydraulic retention, and increasing surface exchange.

As laboratory findings of macrophyte remediation advance into field studies, vegetated wetlands should be quantified for their phytoremediation and natural attenuation capacity (Williams, 2002). It should be noted that field exposures of pesticide runoff into wetlands and ditch structures can be found in recent literature with pesticide remediation quantified through biomonitoring endpoints and chemical analyses (Moore et al., 2002; Schulz et al., 2003a,b; Bouldin et al., 2004a; Cooper et al., 2004). Specific remediation pathways such as rhizo-microbial degradation (Walton and Anderson, 1990; Anderson et al., 1994; Zablotowicz and Hoagland, 1999), soil and sediment interactions (Chung et al., 1996; Maund et al., 2002; Chefetz, 2003; Lalande et al., 2003), and macrophyte-specific pesticide uptake (Jones and Estes, 1984; Hinman and Klaine, 1992; Karen et al., 1998; Lytle and Lytle, 2002) have been investigated as single components of the dynamic processes occurring within these ecosystems. Investigations of these individual pathways enable a better understanding of the phytoremediation capabilities of constructed wetlands and agricultural ditches. It should also be noted that agricultural BMPs such as conservation tillage, cover crops, VFS and riparian zones also incorporate phytoremediation processes. Such techniques are used to minimize pesticide loss from production fields and could be combined with recognized remediation potential of vegetated ditches for a comprehensive phytoremediation strategy within the agricultural landscape.

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