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Phytotoxicity of Atrazine, S-Metolachlor, and Permethrin to *Typha latifolia* (Linneaus) Germination and Seedling Growth

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Abstract Phytotoxicity assessments were performed to compare responses of *Typha latifolia* (L.) seeds to atrazine (only) and atrazine + S-metolachlor exposure concentrations of 0.03, 0.3, 3, and 30 mg L^{-1}, as well as permethrin exposure concentrations of 0.008, 0.08, 0.8, and 8 mg L^{-1}. All atrazine + S-metolachlor exposures resulted in significantly reduced radicle development ($p < 0.001$). A stimulatory effect for coleoptile development was noted in the three highest atrazine (only) exposures ($p = 0.0030$, 0.0181, and 0.0016, respectively). This research provides data concerning the relative sensitivity of *T. latifolia* seeds to pesticides commonly encountered in agricultural settings, as well as critical understanding and development of using *T. latifolia* in phytoremediation efforts for pesticide exposures.

Keywords Pesticides · Seed · Broad-leaf cattail

Typha latifolia (L.), the broad-leaf cattail, is an obligate perennial wetland plant with a diversity of uses. Native Americans utilized the cattail for food, medicine, weaving material, and even down for insulation and lining baby cradleboards (Murphy 1959). While some have suggested using *T. latifolia* as a potential bioenergy crop, large scale efforts have yet to materialize (Dubbe et al. 1988). In addition to these ethno-botanic and potential biofuel uses, *T. latifola* is a popular plant used in phytoremediation efforts of organic and inorganic pollutants (Wilson et al. 2000; Shardendu et al. 2003; Amaya-Chávez et al. 2006; García-Lledó et al. 2011).

Phytotoxicity is the impact a compound causes on plant characteristics such as germination, growth rate, and radicle and coleoptile development. Ecotoxicological studies examining phytotoxicity of organic contaminants are less prominent than those which focus on vertebrates and invertebrates (Wang and Williams 1988). The use of seed germination and root elongation, as well as the ability of *T. latifolia* to serve as a representative aquatic macrophyte species for phytotoxicity assessments have been promoted by Wang (1987), Wang and Williams (1990), and Moore et al. (1999).

First used in 1959, the triazine herbicide atrazine (2-chloro-4-ethylamine-6-isopropylamino-*S*-triazine) consistently ranks as one of the most commonly used herbicides in the US. From 1992 to 2002, US application of atrazine increased 6 % nationwide to nearly 35 million kg (Gianessi and Reigner 2006a). In 2002, atrazine ranked second only to glyphosate in kilograms of active ingredient applied per year among all herbicides in the US (Gianessi and Reigner 2006a). Atrazine is also an effective ingredient in mixtures with other herbicides, such as the chloroacetanilide S-metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-*N*-((1S)-2-methoxy-1-methylethyl) acetamide] which is sold under the trade name Bicep^{TM}. S-metolachlor, which contains 88 % of the S-isomer as opposed to 18 % of the R-isomer, is a popular herbicide in its own right, accounting for 6 % of the US herbicide usage in 2002 with 11.3 million kg applied (Gianessi and Reigner 2006a). A third generation pyrethroid, permethrin [3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate] is a blend of the *cis*- and *trans*-stereoisomers and was first registered (conditionally) in 1979 as a cotton insecticide. Approximately 266,000 kg of permethrin active

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ingredient were applied in the US in 2002 (Gianessi and Reigner 2006b).

Expected environmental concentrations (EECs) of atrazine, metolachlor, and permethrin in surface water are variable depending on application rates, geographical location, and climatic conditions. Peterson et al. (1994) reported atrazine and metolachlor EECs in Canada of 2.667 and 3 mg L^{-1}, respectively. Midwestern US stream sites were sampled for both atrazine and metolachlor, with reported median values of 3.97 and 1.73 μg L^{-1}, respectively (Battaglin et al. 2000). In that same study, maximum measured atrazine and metolachlor concentrations were 224 and 143 μg L^{-1} (Battaglin et al. 2000). Triplett et al. (1978) observed a maximum atrazine concentration of 0.48 mg L^{-1} in runoff occurring soon after pesticide application. Permethrin peak EECs range from 0.20 to 5.32 μg L^{-1} (USEPA 2006).

Because *T. latifolia* is a popular and successful species used for phytoremediation in constructed wetlands and drainage ditches, it is important to determine potential effects of pesticides commonly encountered in such systems. In order to address effects of commonly used pesticides on early growth and establishment of *T. latifolia*, a study was conducted to assess the effects of atrazine, metolachlor, and permethrin on seed germination and growth.

Materials and Methods

Germination and radicle and coleoptile growth effects of *T. latifolia* (L.) seeds exposed individually to Atrazine 4F™ (atrazine), Bicep™ (atrazine + S-metolachlor), and Hi-Yield 38 Plus™ (permethrin) were examined. Commercial formulations of the three pesticides were used in experimental exposures. A range of individual pesticide concentrations was chosen for this study in anticipation of generating exposure–response data. Targeted concentrations for atrazine (only) and atrazine + S-metolachlor were 0.03 mg L^{-1}, 0.3, 3, and 30 mg L^{-1}. Permethrin targeted concentrations were 0.008, 0.08, 0.8, and 8 mg L^{-1}. These ranges of targeted concentrations fall within pesticide EECs and reported field concentrations (Peterson et al. 1994; Battaglin et al. 2000, US EPA 2006). An atrazine pesticide stock solution (1,000 mg L^{-1}) was prepared by diluting 2.09 mL of Atrazine 4F™ in 1 L of deionized (DI) water. An atrazine + S-metolachlor stock solution (1,000 mg L^{-1}) was made by diluting 3.47 mL of Bicep™ in 1 L of DI water. Lastly, a permethrin stock solution (1,000 mg L^{-1}) was made by diluting 2.63 mL of Hi-Yield 38 Plus™ in 1 L of DI water. Individual exposure concentrations were then prepared using aliquots of the 1,000 mg L^{-1} stock and diluting to 1 L with DI water. Aliquots of stock pesticide solutions were analyzed prior to seed introduction.

Inflorescences from *T. latifolia* were collected from plants at the University of Mississippi Field Station, (Abbeville, MS), placed in plastic bags and stored in a freezer at 0°C until use. To obtain seeds, inflorescences were placed in a commercial blender and filled with DI water and blended for approximately 20 s. Seeds which sank were considered viable, while lighter, non-viable seeds floated and were discarded (Rivard and Woodard 1989). Each pesticide treatment consisted of five replicates of 10 individual *T. latifolia* seeds per replicate, for a total of 50 seeds per treatment. Each replicate was prepared by placing 10 seeds in a pre-labeled 100 × 10 mm Kimax® petri dish containing a single Whatman® 90 mm #1 filter. Fifteen milliliters of the appropriate pesticide solution were added to corresponding treatments (pesticide and concentration) and replicates. Each control treatment replicate received 15 mL of DI water, which was analyzed to ensure no pesticide contamination. Dishes were covered with lids and placed in a Powers Scientific Inc. Model DT 70SDF plant growth chamber for seed germination. Temperature in the chamber was maintained at 20 ± 1°C with a 16 h light: 8 h dark photoperiod for 7 days. At the end of 7 days, dishes were retrieved from the growth chamber and three variables were recorded on each seedling: germination, coleoptile (shoot) length, and radicle (root) length.

An Agilent® Model 7890A gas chromatograph (GC) equipped with dual Agilent® 7683B series autoinjectors, dual split-splitless inlets, dual capillary columns, and Agilent® ChemStation was used for all pesticide analyses. The autoinjector volume was set at 1.0 μL fast mode for all targeted pesticide analyses. The GC was equipped with two micro electron capture detectors (μECDs). Ultra-high purity (UHP) helium at 28 mL min^{-1} was the carrier gas, while the inlet temperature was 250°C. With a constant make-up gas flow of 60 mL min^{-1} UHP nitrogen, the μECD temperature was 325°C. Analytical columns used for atrazine and metolachlor were Agilent® HP 5MS capillary columns, 30 m × 0.25 mm i.d. × 0.25 μm film thickness. An Agilent® HP 1MS capillary column, 30 m × 0.25 mm i.d. × 0.25 μm film thickness was used for permethrin. Only the parent compound was analyzed for atrazine and S-metolachlor. Both the *cis* and *trans* isomers of permethrin were determined analytically. Limits of analytical detection in aqueous exposures were as follows: atrazine = 0.001 mg L^{-1}; metolachlor = 0.0001 mg L^{-1}; and permethrin = 0.0001 mg L^{-1}. Percent recoveries for pesticides were ≥94 %.

Germination and radicle and coleoptile growth data were analyzed using a one-way analysis of variance and Student's t-test (atrazine versus atrazine + S-metolachlor) or Tukey–Kramer HSD test (control versus individual

pesticide), with an alpha level of 0.05 (JMP version 8.0 software, Cary, NC).

Results and Discussion

All aqueous test solutions (including control DI water) were analyzed for atrazine, S-metolachlor, and permethrin. None of the three pesticides were detected in the control water. Atrazine 4F™ test target concentrations of 0.03, 0.3, 3, and 30 mg L^{-1} actually measured 0.024, 0.219, 2.527, and 17.52 mg L^{-1} atrazine, respectively. Bicep™ test target concentrations of 0.03, 0.3, 3, and 30 mg L^{-1} measured 0.031, 0.280, 3.021, and 22.31 mg L^{-1}, respectively, for atrazine and 0.027, 0.299, 3.148, and 27.94 mg L^{-1}, respectively for S-metolachlor. Hi-Yield 38 Plus™ test target concentrations of 0.008, 0.08, 0.8, and 8 mg L^{-1} measured 0.004, 0.039, 0.354, and 4.777 mg L^{-1}, respectively, for *cis*-permethrin and 0.003, 0.032, 0.300, and 3.970 mg L^{-1}, respectively, for *trans*-permethrin.

Tukey–Kramer HSD results indicated no significant differences in germination between control exposures and any of the target pesticide concentrations. Germination percentages for this study ranged from 59 ± 9.0 % (atrazine + S-metolachlor 0.03 mg L^{-1} exposure) to 100 ± 0 % (permethrin 4 mg L^{-1} exposure) (Table 1).

Radicle development indicated statistically significant differences between control and pesticide exposures. In the atrazine-only exposures, development of radicles in seeds exposed to 30 mg L^{-1} (target) atrazine were significantly less than radicles in controls ($p < 0.001$). Although different statistical analyses were employed in Moore et al. (1999), that study also reported a decrease in radicle development in higher atrazine exposures. In atrazine + S-metolachlor exposures, radicle development was significantly less than controls in all four exposure concentrations ($p < 0.001$) (Table 1). A stimulatory effect for radicle development was noted in permethrin target exposures of 0.8 mg L^{-1}. A significant increase in radicle development was noted in this target concentration when compared to control ($p < 0.001$)(Table 1). Tu (1982) reported that permethrin had neither toxicity nor inhibitory effects on the growth of soybeans (*Glycine max*). Cypermethrin, however, decreased *Pakchoi* seed root elongation with increasing concentration from 5.6 to 17 mg L^{-1} (Liu et al. 2009). Additionally, all four exposure concentrations demonstrated significant decreases in radicle development when comparing atrazine (only) exposures to atrazine + S-metolachlor exposures ($p < 0.001$).

Coleoptile development varied from that observed in radicles exposed to pesticides in the current study. For atrazine-only exposures, no statistically significant differences were noted in coleoptile development when compared to controls; however, all three exposure concentrations resulted in a stimulatory effect (Table 1). This was expected since atrazine's mode of action is a photosystem II inhibitor. Esser et al. (1988) reported that triazine herbicides, such as atrazine, stimulate plant shoot length, leaf blade, and stem diameter. Burhan and Shaukat (2000) reported atrazine's phytotoxicity to pearl-millet (*Pennisetum americanum* L. Schumann), wheat (*Triticum aestivum* L.), turnip (*Brassica napobrassica* Mill.), carrot (*Daucus carota* L.), corn (*Zea mays* L.), and mustard (*Brassica compestris* L.). In examinations of both root and shoot length, turnip was affected the most by atrazine, while mustard was the least affected (Burhan and Shaukat 2000). In atrazine + S-metolachlor exposures, coleoptile development was significantly different than that observed in control coleoptiles for the 3 and 30 mg L^{-1} targeted exposures ($p < 0.001$ and $p < 0.001$, respectively). When comparing atrazine (only)

Table 1 Seed germination and mean radicle and coleoptile growth of *T. latifolia* L. exposed to pesticides (n = 40 seeds per treatment) after 4 days treatment exposure

Treatment	Mean ± SE		
	Germination (%)	Radicle (mm)	Coleoptile* (mm)
Control	85 ± 5^{a,b,c}	5.82 ± 0.33^{b,c}	6.84 ± 0.24^{a,b,c}
ATZ (0.03 mg L^{-1})	66 ± 10^{b,c}	5.89 ± 0.35^{b,c}	7.29 ± 0.26^{a,b,c}
ATZ (0.3 mg L^{-1})	80 ± 5.4^{a,b,c}	5.50 ± 0.23^{c}	7.74 ± 0.19^{a,b}
ATZ (3 mg L^{-1})	82 ± 5.6^{a,b,c}	6.47 ± 0.82^{b,c}	7.55 ± 0.31^{a,b}
ATZ (30 mg L^{-1})	91 ± 4.1^{a,b}	3.75 ± 0.07^{d}	7.79 ± 0.21^{a}
ATZ/MET (0.03 mg L^{-1})	59 ± 9.0^{c}	1.34 ± 0.06^{e}	7.70 ± 0.22^{a,b}
ATZ/MET (0.3 mg L^{-1})	85 ± 2.1^{a,b,c}	1.00 ± 0.00^{e}	6.83 ± 0.25^{b,c}
ATZ/MET (3 mg L^{-1})	88 ± 3.7^{a,b,c}	0.73 ± 0.16^{e}	3.28 ± 0.34^{d}
ATZ/MET (30 mg L^{-1})	80 ± 8.1^{a,b,c}	0.00 ± 0.00^{e}	1.00 ± 0.00^{e}
PERM (0.008 mg L^{-1})	66 ± 8.2^{b,c}	5.95 ± 0.76^{b,c}	7.33 ± 0.20^{a,b,c}
PERM (0.08 mg L^{-1})	81 ± 6.2^{a,b,c}	6.91 ± 0.44^{a,b}	7.72 ± 0.26^{a,b}
PERM (0.8 mg L^{-1})	78 ± 8.5^{a,b,c}	8.38 ± 0.72^{a}	6.94 ± 0.38^{a,b,c}
PERM (8 mg L^{-1})	100 ± 0.0^{a}	5.42 ± 0.48^{c}	6.48 ± 0.20^{c}

Target pesticide concentrations are in parenthesis. Levels in columns not connected by the same letter are significantly different

ATZ atrazine, *ATZ/MET* atrazine + S-metolachlor, *PERM* permethrin

* ATZ (0.3 mg L^{-1}) and ATZ/MET (0.3 mg L^{-1}) were significantly different from each other based on the Student *t* test

to atrazine + S-metolachlor exposures, significant differences in coleoptile development were noted at target concentrations of 0.3, 3, and 30 mg L^{-1} ($p = 0.0014$, $p < 0.001$, and $p < 0.001$, respectively). This is not completely unexpected, since metolachlor is a long chain fatty acid inhibitor whose mode of action will affect seedling growth.

Additional research examining pesticide effects on seed germination and root and shoot development of non-target plants, especially those plants used in phytoremediation, is needed. Current results demonstrate significant differences observed in phytotoxicity of single-chemical herbicides versus herbicide mixtures. This information is valuable to conservationists and other practitioners involved in water quality improvement from pesticide runoff exposure.

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